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Volume 38

Edited by
P. H. GREGORY and G. C. AINSWORTH

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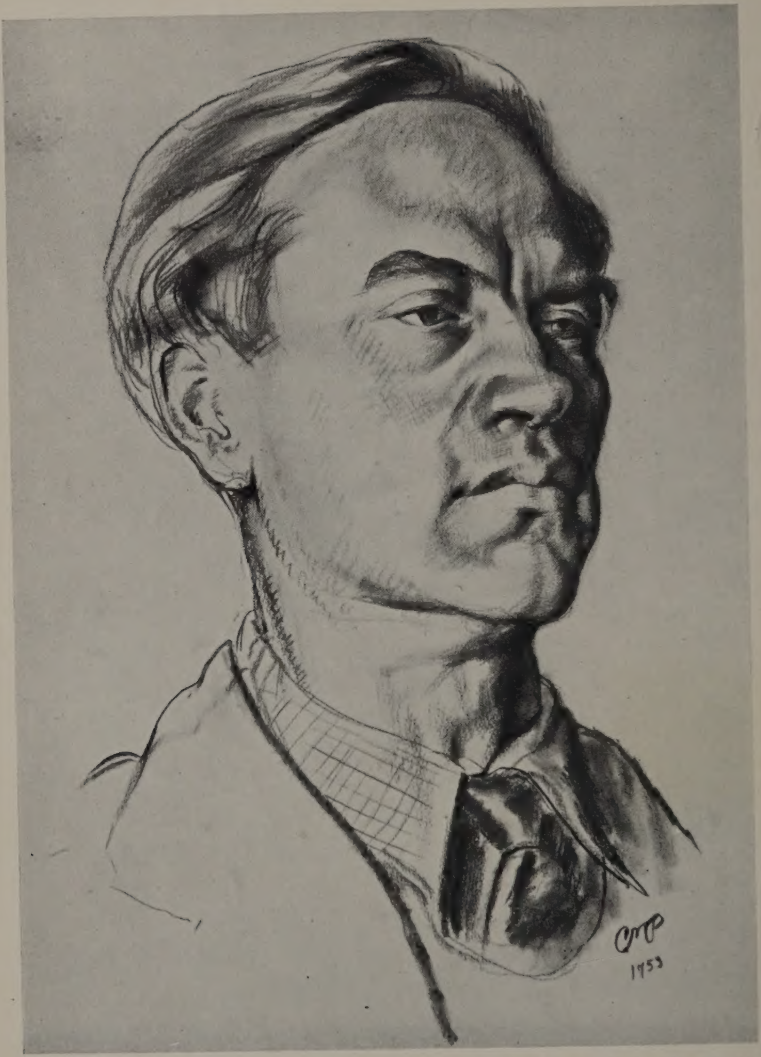

From the drawing by Christopher Perkins

Dr S. D. GARRETT
President, 1954

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PRESIDENTIAL ADDRESS

MICROBIAL ECOLOGY OF THE SOIL

By S. D. GARRETT

Two of my distinguished and most recent predecessors in the office of President have addressed you on the subjects of fungi in air and in water, respectively, and now I am going to speak about fungi, amongst other micro-organisms, in the soil. In four years, therefore, we shall have had Presidential Addresses concerned with no less than three of those four elements distinguished by the ancient philosophers—earth, water, air and fire—of which, in varying proportions, all matter was at one time thought to be composed. According to this now discredited theory, we three protagonists of the three elements earth, water and air might fittingly be characterized by the temperaments held to be associated with these elements. But whereas I may not be too obviously miscast for the role of melancholic type, Prof. Ingold is certainly not a phlegmatic and one could scarcely imagine anyone less choleric than Prof. Gregory. Nevertheless, some improvement in the goodness of fit could be effected if we elect as our next President someone of obviously sanguine temperament who has specialized on thermophilic fungi.

My allotted span of time for this Address now compels me to jump across four intervening centuries into the immediate present. Microbiology to-day is in the era of the microbiological biochemist. As if his spectacular successes in unravelling the biochemical and physiological mysteries of the individual microbial cell were not enough, his prestige has been much enhanced by the immense practical success, no less than by the fundamental scientific interest, attending the discovery and exploitation of microbial antibiotics during the past two decades. In this connexion, the names of Fleming, Florey and Waksman have rightly become household words. The microbiological biochemist views the microbial cell as a miraculous factory for the performance of chemical syntheses. He can tell us under what conditions, *in vitro*, this miniature factory carries out certain chemical syntheses, and under what conditions it performs such syntheses most 'efficiently', i.e. with a maximum yield of the final product. Faced by the great and still growing prestige of the microbiological biochemist, and further dazzled by the related work of the microbial geneticist, the general microbiologist trained as a botanist is apt to feel somewhat eclipsed. If, like myself, he is unwilling to abandon the tools of his own trade, and to seek proficiency in the use of these newer ones, he may well ask what is there left for him to do? There is, however, no need for him to feel this sense of inferiority. The answer to my question has already been given by a greater mycologist than I; as Prof. William Brown has

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remarked in his Foreword to the well-known book, *Physiology of Fungi*, by Dr L. E. Hawker (1950): 'In the last resort, the greatest scientific interest concerned lies in the functioning of the organism as a whole.' In other words, the mycologist trained as a botanist is concerned with the behaviour of the whole fungus in relation to its environment. His equipment and techniques are quite adequate for the discovery and further elucidation of physiological phenomena; for further analysis on the biochemical level, however, the mycologist trained only as a botanist must work in collaboration with a biochemist, or else be content to make other explorations on his own level, for which there is ample scope far into the foreseeable future.

As I have just said, the mycologist as such is concerned with the behaviour of the whole fungus in relation to its environment. In my own sphere, that of soil microbiology, the most important part of the environment for any fungus or other micro-organism consists of the living population of other soil micro-organisms. It is here that the botanically trained soil microbiologist comes into his own. In this respect, soil microbiology is simply a branch of ecology—it is concerned with the inter-relationships of organisms occupying a common habitat, or rather a series of habitats. The plant ecologist studying vegetation above ground endeavours to unravel the complex inter-relationships between individuals and between groups of higher plants forming an association or community. The habitat determines the vegetation, but the vegetation in its turn changes the characters of the habitat, as can be seen in autogenic succession, in which each of a series of transitional plant associations further improves the habitat for growth of more specialized and demanding species, until the climax association is reached. The phenomenon of autogenic succession can similarly be seen on a microcosmic scale within the soil, in the colonization and consumption of a substrate by soil micro-organisms, though in this instance of it the succession of organisms does not improve but rather depletes the capacity of the habitat to support further plant life, so that the end-point of a succession of micro-organisms on a substrate is not a persisting climax association, but zero.

A number of such interesting and perhaps fruitful analogies can be drawn between the ecology of higher plants and the microbial ecology of the soil. Acquaintance with the concepts and techniques of plant ecology can thus help the soil microbiologist in his study of inter-relationships between groups of soil micro-organisms and their habitat. The aims and methods of the microbiological biochemist do not fit him to deal with this aspect of the problem; his techniques, for the most part, have been designed for the pure-culture study of micro-organisms *in vitro*. The techniques of the microbiological biochemist and those of the microbial ecologist are thus strictly complementary.

Now in stressing the value of the ecological approach to problems of soil microbiology, I do not wish to imply that this approach is in any sense a new one for soil microbiologists. The outstanding contributions to microbial ecology of such great men as Winogradsky would be a living reproach to any such assumption. Still less do I wish to let it be inferred that all or even many of the methods and techniques of higher plant ecology can be applied directly to the investigation of soil microbiology. The value of a technique is not limited by the scope of its immediate, or even of its modified, practical application. Its suggestive value may ultimately exceed its immediate practical value, and it is in this way that one branch of ecology can most help another. Techniques are indeed all-important for the advance of any science. Sometimes the advent of a new technique produces a powerful impetus to fresh research. In soil microbiology, one could instance here the enrichment culture method of Beijerinck and the Rossi-Cholodny soil slide method for direct observation of the soil microflora. At other times, new concepts arise out of pre-existing knowledge, and demand new techniques for the fulfilment of their promise. About the pressing need for new techniques of investigation in microbial ecology I shall have to speak hereafter.

THE SUBSTRATE PATTERN OF MICROBIAL DISTRIBUTION

In 1904 Hiltner announced his discovery of the rhizosphere—a zone of intensified microbial activity around the growing roots of higher plants. It is now known that the rhizosphere flora is not only quantitatively but also qualitatively different from that of soil remote from roots. The effect increases in intensity with closer approach to the root surface; in soil actually in contact with roots, total numbers of bacteria may be 100 times those in soil remote from roots. Although various mechanisms have been postulated to account for this effect, it seems likely that intensified microbial activity is chiefly due to production of substrates by the growing root, in the form of dead cells and tissues sloughed off during growth, and of organic substances continuously excreted into the adjacent soil.

We can now realize that Hiltner's rhizosphere effect is, in fact, only a particular instance of a more general substrate effect on the soil microflora. Substrates for soil micro-organisms consist of living or dead, virgin or partially decomposed, plant or animal tissues lying in or upon the soil, and of soluble products diffusing therefrom. Any substrate present in or on the soil that becomes accessible to utilization by micro-organisms must go through a series of phases, viz. colonization, exploitation and exhaustion. During this process, a succession of soil micro-organisms develops upon the substrate; the precise sequence of micro-organisms must be determined both by the nature of the substrate and by the environment of the microhabitat. The peculiar characteristics of population behaviour in microorganisms, and their relationship to the nature of substrates, have recently been clarified by Stanier (1953), in an exposition of outstanding interest.

Some soil microbiologists have questioned the occurrence of an ordered succession of soil micro-organisms on a substrate constituting a microhabitat within the soil, because of the technical difficulties of demonstrating such a succession. The difficulties and labour attendant upon the identification of soil bacteria need no elaboration here. Even with soil fungi, which can usually be identified by their morphology alone, direct microscopical observation of a substrate in process of colonization can permit identification only of those occasional species that happen to produce recognizable spore-forms *in situ*. Sampling for identification by means of the agar isolation plate, on the other hand, is seriously biased by the selectivity of all known isolation methods. A yet more awkward complication is that colonies of fungi developing on the isolation plate may have come either from currently active mycelium or from resting bodies produced by fungi active during an earlier stage of the succession. Any sample taken from such a substrate undergoing decomposition may therefore give, on the isolation plate, colonies of fungi representing any or all of the earlier stages of fungal succession on the substrate. This difficulty is evaded in the common laboratory demonstration of the sequence of fungal fruit bodies developing upon dung incubated moist under a bell-jar. The number of fungi forming fructifications upon the dung, however, is likely to be but a fraction of the total number of species engaged in successional decomposition of the substrate.

A new picture of the distribution of soil micro-organisms is thus slowly emerging. We may imagine the soil as a three-dimensional pattern of substrates, each of which passes in its turn through the successive phases of colonization, exploitation and exhaustion. Sites of former substrates, now exhausted, are marked by the resting cells of organisms that had colonized them, at least for as long as the resting cells survive. This simple conception seems adequate to account for the distribution of most soil bacteria, or at least for that of non-motile forms. For soil fungi, there is an additional complication in the potential capacity of some fungi for making outwards growth from a colonized substrate through the surrounding soil. Nevertheless, it seems likely that many fungi, and perhaps the majority of saprophytic sugar fungi, may behave somewhat like non-motile soil bacteria, forming resting cells upon the substrate rather than growing out as mycelium through the soil. It is certainly possible for fresh substrates to come to a resting fungus in this way, by the growth of plant roots and by the migration of small animals through the soil. After consumption of a substrate, a fungus can devote its accumulated food reserves either to production of resting bodies or to outwards mycelial growth through the surrounding soil. In the first way, the fungus extends its duration in time; in the second, its distribution in space. These two alternatives are not mutually exclusive for any one fungus at any time, but one must occur at the expense of the other, because the food reserves at the disposal of the fungus are limited by the capacity of the substrate.

Granted acceptance of this substrate-pattern of micro-organisms throughout the soil, and indeed the existence of such a pattern seems a self-evident truth, then it will be equally clear that present techniques of sampling for investigation of the soil microflora are inadequate for further advances in our understanding of microbial ecology. Work on the fungus flora of the soil has already resulted in the compilation of extensive floristic lists for different types of soil. Although all isolation procedures are admittedly selective, this disadvantage has been reduced by the variety of procedures employed; these include the 'baiting' of soil with special substrates designed for the isolation of particular groups of fungi, as first introduced by Butler (1907) for the study of *Pythium* species. The substrate for colonization is similarly introduced into the soil by the immersion tube method of Chesters (1940). Floristic lists compiled in this way have told us much about the variation of the fungus flora from one soil to another, and from one horizon to another in the same soil. Without more precise methods of sampling, however, we can expect no further information than this. Relatively crude methods of soil sampling may be adequate for a study of the distribution of higher plants, because a growing plant summarizes by its development the variety of soil conditions encountered within its root range. For micro-fungi at least, the effective mycelial range of a single fungus colony from a substrate within the soil is very much smaller than the root range of a higher plant. In the taking of soil samples for isolation of micro-fungi, the samples have usually been taken carefully at random, and have frequently been bulked and thoroughly mixed before plating so as to obtain a 'representative' sample. This method has been adequate, and perhaps even essential, for the compilation of floristic lists of fungi from different types of soil at different depths and from different regions of the world. In the very act of taking such soil samples, however, the substrate pattern of fungi within the soil has been destroyed. Such methods of investigation have thus given some idea of the species, and of the frequency of species, of fungi in a particular type of soil, and at different depths in that soil, but little further information as to distribution. The limitations of such a technique can best be appreciated by supposing that an ecologist studying the vegetation of a meadow had to confine his observations to samples of herbage taken from a haystack.

It is relevant here to cite the views of Stanier (1953) upon the distribution of micro-organisms in nature; the proverbial nutshell is almost too gross a receptacle to serve as a simile for the compression of ideas in the following passage:

> The microbial environment is both wider and narrower than that of the plant or animal. It is wider in the sense that there are usually no *geographical* limitations to the distribution of a species, except in the case of parasites, whose distribution is governed by that of their host organism. As far as free-living micro-organisms are concerned, a very broad ecological classification into terrestrial, marine and fresh-water forms is generally possible, but localization on a macroscale can go no further.* The reason for this well-nigh universal distribution is that microbial environments are micro-environments, hundreds or even thousands of which lie concealed from the gross ecological eye in any gram of soil. A single cellulose fibre provides a specialized environment with its own characteristic microflora, yet may occupy a volume of not more than a cubic millimetre. Such environments are omnipresent, and do not differ significantly whatever their geographical location.

So far as the soil fungi are concerned, and it is these on which I am myself best qualified to speak, it is the similarities rather than the differences between floristic lists compiled in different regions of the world that are most impressive. As Waksman was the first to emphasize from 1916 onwards, the fungus flora of the soil is essentially a cosmopolitan one. This comparative constancy of the soil fungus flora as compared with the more obvious ecological variation in the above-ground flora of higher plants in different regions of the world can be attributed partly to the fact that the soil environment is physically better buffered than the subaerial environment, so that organisms do not suffer such extremes of temperature and drought. It is also true that micro-organisms in general are more tolerant of environmental extremes than are higher plants; as we ascend the evolutionary scale, tolerance of unfavourable conditions tends to diminish. This tolerance of micro-organisms to environmental extremes may be attributed in part to their capacity for prolonged dormancy. Stanier has remarked upon this capacity as an essential characteristic of microbial populations, and has related it to the fact that substrates for micro-organisms, in contrast to most habitats for higher plants, are discontinuous in time. Secondarily, however, such a capacity for dormancy also permits a wide tolerance of environmental extremes, and results in a geographical distribution that is always wide, and usually world wide.

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* Stanier has qualified this in the following footnote: 'This statement may not be wholly valid for fungi. Emerson (1941) has shown that the free-living phycomycete *Allomyces*, a soil inhabitant, occurs only in tropical and warmer temperate regions (approximately latitude 40° N. to 40° S.); within this band, distribution is world-wide.'

THE NEED FOR NEW TECHNIQUES

We can now realize that the substrate-fungus relationship is the key to the distribution of fungi within the soil. To stress the central importance of this relationship is not to underestimate the achievements of earlier mycologists, whose work has indeed made possible this clarification. Apart from our quite extensive knowledge of the gross distribution of fungi in different soils, much is known about the substrates of different soil fungi from general observation, from soil-baiting procedures, and from biochemical determinations of capacity to decompose various carbon substrates, including cellulose and lignin, in pure culture. For further investigation of this substrate pattern of fungi in the soil, several approaches are possible. First, improved techniques are required for studying colonization of natural substrates by soil fungi, for determining the succession of fungi developing on such substrates, and for observing the subsequent behaviour of fungi after colonization and consumption of a substrate. These requirements, though easy to state, will be difficult to fulfil. Direct observation methods give little information as to identity of the fungi observed; cultural isolation methods are selective, and also fail to discriminate between currently active mycelium and resting bodies. It seems likely that no single method can be devised to give all the required information, and that a number of complementary techniques will have to be employed jointly. Difficulties such as these have limited the value of the information obtained, for instance, by various workers who have studied fungal colonization of plant roots, from senescence onwards. A second type of approach to this general problem is favoured by those who are more experimentally minded; suitable substrates are placed in the soil and their colonization is then followed. This method has the advantage of simplifying the problem, inasmuch as chemically defined substrates of different types can be employed. A particularly ingenious use of this technique was made by Timonin (1941), who constructed root models out of collodion sacs filled with root exudate, for studying the rhizosphere microflora.

Yet a third type of approach to problems of behaviour and distribution in the soil fungi has been made by those who, like myself, have been mainly preoccupied by the root-infecting fungi. This is the autecological method, whereby a single organism is studied as intensively as possible in relation to its habitat. It is interesting to recall here the words of an eminent pioneer of plant ecology; Sir Arthur Tansley (1923) has declared: 'The "ideal" method of study might be to investigate each species separately, till we knew in detail its life history, the methods by which and the rate at which it could spread, its behaviour under different conditions of climate and soil, and only when we had obtained this knowledge proceed to study the species as it existed in communities along with other species.' This method, which thirty years ago Tansley thought to be ideal but impracticable, has now proved its value for the study of organisms of great economic importance, such as root-disease fungi and pasture plants, which grow or are grown in natural or semi-natural associations with other species. This thorough autecological study of individual species, which Tansley declared to be one of the greatest needs of ecology, has in fact been carried out with a few root-infecting fungi, as well as with some other soil micro-organisms of great practical importance, such as the Rhizobia of legume nodules. Where a single micro-organism alone is concerned, special techniques can be devised for its direct or indirect observation in the natural or semi-natural soil environment; highly selective techniques for detection or isolation of the micro-organism, such as the seedling host test, can be employed and no difficulties comparable to those of studying the whole fungus flora at one time are encountered. Such autecological studies of root-infecting fungi contrast with work on general soil fungi in their much wider use of the experimental approach; by the soil tumbler method and its various modifications, laboratory study of fungal behaviour under strictly defined soil conditions has been made possible.

Lest it be thought, however, that I am exalting my own particular branch of soil microbiology at the expense of others, I will hasten to add that the plant pathologist's approach to soil microbiology has also been lopsided. For many years preoccupied with the host-parasite relationship to the virtual exclusion of the other soil micro-organisms in the root environment, the plant pathologist is still too much obsessed by the effect of disease on the host plant, to the comparative neglect of fungal behaviour in the absence of the host. For a number of root-infecting fungi, we are now tolerably well informed about infection behaviour of the fungus and about development of the disease under a wide range of environmental conditions. By comparison, our information about the production and survival of fungal resting bodies is scanty, and that about saprophytic survival of root parasites in the actual soil is equally sparse. It is their saprophytic behaviour in the soil that is still the hidden phase in the life cycle of most root-infecting fungi. There is a natural tendency for pathologists, whether concerned with plant or with animal disease, to identify themselves figuratively with the host side of the host-parasite relationship. To overcome this frankly emotional bias, it is an essential intellectual exercise for the pathologist to view disease in turn from the side of the micro-organism, with which he must mentally associate himself. Seen from this angle, the host-parasite relationship wears a new aspect, appreciation of which is essential for complete understanding of possible trends in parasitic evolution. Such a revelation can be found in a book of unique value for all pathologists; I refer to Sir Macfarlane Burnet's *Natural History of Infectious Disease*, of which a new edition was published in 1953. Burnet has himself epitomized his book in a notable aphorism concerning 'the outlook of a biologist, to whom both man and micro-organism are objects of equal interest'.

EPILOGUE

In what I have said in this Address, I have tried to express my view that, for the biologist, soil microbiology is nothing if not a branch of ecology. Although to some soil microbiologists, this must always have been an obvious truth, yet there are times when we need to be reminded of such obvious truths as the essential unity of the biological sciences. Most of us must be acutely aware of the difficulty in which the increasing specialization of biology, and indeed of science in general, places us. In order to get to the frontiers of knowledge in our own subject, let alone to extend them, we have to specialize as never before. For the microbiologist, the microscope is all too symbolic of the intense but narrow direction of vision that is becoming necessary. Such specialization makes for isolation of biologists one from another; a common understanding is an essential requisite for healthy controversy, without which science loses its vitality, and dogma can masquerade as truth.

At the outset of his career, the young biologist shortly to become a research worker receives, we may hope, a liberal education in biology in the final stages of a liberal general education. It will be well for him if he has done so, because after that, as a research worker, it becomes increasingly difficult for him to look anywhere except straight ahead for quite a number of years. At length, if he is fortunate, he has time in his middle and later years to look around once more—in other words, to re-educate himself. This time, informed by experience, education will mean much more to him, and he may be able to effect an intellectual synthesis between his own work and that in other fields of human endeavour. I should like to quote here a short passage from a remarkable book for biologists by Mrs Agnes Arber, *The Mind and the Eye* (1954):

> When we try to trace the concept of wholeness, as it develops in a man's mind, we realize that, as an infant, his vision of the surrounding world possesses a certain primitive unity, since, in a sense, it forms a whole, not yet discriminated into components. At the opposite pole is the kind of unity achieved by mature thought, in which fully analytical observation of individual things, and the differentiation of individual ideas, has been followed by a synthesis which has reconstructed unity from diversity. Between these two poles—the first, unconscious, and the second, self-conscious—lies the whole developmental sequence of the intellectual life.

In the light of Mrs Arber's observations in this passage, the increasing specialization that has come to be necessary for the biological research worker, at least during one phase of his life, can be seen as an essential formative influence for the mature biologist. Without the specialization that intense concentration demands, 'fully analytical observation of individual things, and the differentiation of individual ideas' can never be achieved, and an essential step in intellectual experience will not have been taken. Without this experience, a mature synthesis of biological ideas, which is the final stage in the development of a biologist, can never be reached. For the intense joy which this mature synthesis may bring, the words of Sir Charles Sherrington from *Man on his Nature* (1951) are perhaps more apt than any others: 'We dismiss wonder commonly with childhood. Much later we may return.'

REFERENCES

ARBER, A. (1954). *The mind and the eye.* Cambridge University Press.
BURNET, F. M. (1953). *Natural history of infectious disease.* Cambridge University Press.
BUTLER, E. J. (1907). An account of the genus *Pythium* and some Chytridiaceae. *Mem. Dep. Agric. India, Bot.* **1**, no. 5.
CHESTERS, C. G. C. (1940). A method of isolating soil fungi. *Trans. Brit. mycol. Soc.* **24**, 352–355.
HAWKER, L. E. (1950). *Physiology of fungi.* London: University of London Press.
SHERRINGTON, C. (1951). *Man on his nature.* Cambridge University Press.
STANIER, R. Y. (1953). Adaptation, evolutionary and physiological: or Darwinism among the micro-organisms. *Adaptation in micro-organisms. Third. Symp. Soc. gen. Microbiol.* Cambridge University Press.
TANSLEY, A. G. (1923). *Practical plant ecology.* London: George Allen and Unwin.
TIMONIN, M. I. (1941). The interactions of higher plants and soil micro-organisms. III. Effect of by-products of plant growth on activity of fungi and actinomycetes. *Soil Sci.* **52**, 395–413.

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MYCOSES OF THE BELGIAN CONGO*

By R. VANBREUSEGHEM

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As regards mycoses, the Belgian Congo does not differ from other countries. I could speak for a long time about dermatomycoses and the dermatophytes, chromoblastomycosis and torulosis, histoplasmosis, Tinea versicolor, moniliasis and Madura foot, but many of these topics are too specialized to be of general interest, and so I propose to touch on certain wider aspects of a selection of these mycoses.

DERMATOMYCOSES

We have recorded a fair number of dermatophytes, known or unknown, from the Belgian Congo, and we have noticed that their distribution within the country differed from one region to another. The fact that the true Trichophytons are more prominent in the eastern hilly parts of the Congo and that the Microsporums are more frequently encountered in the central basin is paralleled in other parts of the world. That climate and surroundings have something to do with the distribution of fungi is sometimes very clear, for example, *Histoplasma capsulatum* and *Coccidioides immitis*. It may not be so clear for the dermatophytes, although from the very beginning of medical mycology, eminent dermatologists have insisted on the regional character of the dermatophytic flora. I, too, have come round to this point of view, and I have frequently insisted that each region possesses a characteristic spectrum of dermatophytes. It is not easy to understand the reason why a dermatophyte so common in Europe as *Microsporum canis* or a *Trichophyton* so frequently encountered in North Africa as *T. schoenleini* has not yet been isolated from the numerous cases of dermatophytia capitis or dermatophytia corporis in the Belgian Congo. Why, when a boy with a Tinea capitis caused by *Microsporum audouini*, is introduced into North Africa, is there no epidemic? Or, why, when small epidemic foci are created by the introduction of persons infected with *Trichophyton ferrugineum* or *T. soudanense* into temperate countries, as happened lately in the Paris region, do they not spread?

I have previously expressed the hypothesis that a part of the normal life cycle of dermatophytes takes place in the soil from where new epidemics originate (Vanbreuseghem, 1952*a*). I also expressed the hypothetical view that the regional spectrum of dermatophytes would depend upon the nature of the soil (Vanbreuseghem, 1952*b*). Consequently, one might expect that some dermatophytes would be of world-wide distribution, and that the distribution of others would be more restricted. This is of course what I would like to call in French, a pure 'vue de l'esprit', although for my first hypothesis it must not be forgotten that some dermatophytes—*Microsporum gypseum* and *Keratinomyces ajelloi*—have been isolated from soil. What about the others? That is another question which still awaits a full answer.

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* Address given on 6 April 1954 at a meeting of medical and veterinary mycologists arranged by the Medical Mycology Committee of the Medical Research Council in the Department of Dermatology, Leeds General Infirmary.

TINEA VERSICOLOR

Now, a few words about a strange skin disease, so common in the Congo that nearly every native is a living illustration of it: I speak of the Pityriasis versicolor, possibly better known here as Tinea versicolor. The first point of interest is that the disease is so common in the Belgian Congo that every native is either afflicted by these skin blemishes or has been, or will be, a victim of this mycosis. The Europeans living in the Congo do not seem more commonly to suffer from Tinea versicolor than permanent residents of France or Belgium. The second point is that whereas in France or Europe the face usually escapes infection, the brownish, whitish and slightly scaly patches typical of Tinea versicolor are very common on the face of the Negro (Vanbreuseghem, 1950). I have even found some cases of scalp infection among them, an occurrence which is quite exceptional in Europe. The third point, of special interest for the mycologist, is that the microscopical morphology of *Malassezia furfur* is the same in the Congolese as in the European cases of Tinea versicolor. It is always the groups of double-contoured spores and the fragmented mycelium which forms a basis for microscopical diagnosis. I have frequently insisted that we cannot always conclude from an identical parasitic morphology the identity of the pathogenic fungi. This fact is very clear for the dermatophytes where the most undisputed species produce an identical aspect in the hair (*Microsporum canis*, *M. audouini* and *Trichophyton ferrugineum*). It is equally true for *T. violaceum*, *T. sabouraudi*, and for a parasite so different morphologically that I felt justified in creating for it a new genus, *T.* (*Langeronia*) *soudanense*. The conclusion is evident; as our knowledge of *Malassezia furfur* is purely based on its parasitic morphology, we cannot conclude that a single fungus is responsible for Tinea versicolor. *M. furfur* has never been cultivated with certainty—many unsuccessful attempts have been made in my own laboratory by Lejeune (1952), and Vanbreuseghem and de Tiège (1952)—and we are not far from the truth by saying that *M. furfur* is a nomen nudum. The hypothesis expressed by Gordon (1951) that the cause of Tinea versicolor could be a new species of *Pityrosporum*, namely *P. orbiculare*, has not been confirmed.

Recently, a new technique has been proposed by J. A. Porto (1953) for the diagnosis of Tinea versicolor. A small piece of 'scotch tape' is first applied on the suspect patch and then transferred to a microscope slide. The examination with low- or high-power objectives reveals the typical groups of spores and the mycelia. I do not think that this technique is any better for the diagnosis than the usual scrapings, but I thought that by painting the skin with a strain before the use of the tape, the technique could be useful for a better understanding of the morphology of *Malassezia furfur*. My young collaborator, Doupagne, now in the Belgian Congo, has sent me some of these preparations obtained after painting the skin with an alcoholic solution of eosine (2 %), or with Lugol's iodine solution. Although my final conclusions are still in suspense, I think I understand the morphology of the parasite better. It seems that on the more superficial layers of the epidermis, there are only septate, branching, flexuous hyphae, whose diameter is quite irregular. Very often the mycelium is broken in very small pieces. This mycelium seems to penetrate in a somewhat deeper layer of the more superficial epidermal cells. It is there that the morphology of the sporophores can be observed and an idea obtained of the formation of the spores. The sporophore is branched or unbranched. It is a rather long fusiform piece of mycelium with a cup-like end, very similar to a phialide. The terminal part of the fusiform sporophore bends gently on itself, producing a curve which seems to be a specific character. The spores are blown through the opening of the cup-like process and are deposited in a grape-like manner around the opening and often in the very curve made by the sporophore. From time to time one of the spores buds a daughter-cell, but in no case have I found a daughter-cell as big as the mother-cell. The spores seem to be embedded in some slimy substance which keeps them together. These results seem to point to the classification of the parasite of Tinea versicolor among the fungi reproducing themselves by phialospores (Vanbreuseghem, 1954).

Histoplasmosis

Another problem to which I would like to draw attention is African histoplasmosis and *Histoplasma duboisii*. The solution, which is not yet complete, owes much to the work of your countryman, Dr J. T. Duncan. I had already noted in the second edition of the *Précis de Mycologie* (Vanbreuseghem, 1952*c*), which I completed after the death of my master, Maurice Langeron, that histoplasmosis presented itself as a peculiar problem in Central Africa. My reasons were as follows: the symptomatology of the African cases was somewhat special and characterized by cold abscesses, but, and this fact had not received all the attention it deserved except by Duncan (1947), the parasites in the African cases were rather different from that of the classical histoplasmosis. It is now known that in African histoplasmosis, *Histoplasma duboisii* is seen in the tissues as large ovoid cells, with a very thick cell-membrane and many droplets of fat dispersed in the protoplasm. From my own point of view, the most characteristic and most stimulating fact about this parasite manifests itself when you inoculate a guinea-pig by the intra-testicular route with a culture of *H. duboisii*. If a week or two after this inoculation the testicle is examined, you can already observe many parasites which have not the typical morphology of *H. duboisii* in the tissues but that typical of *H. capsulatum*: to this particular aspect which characterizes the invasion of the host by *H. duboisii*, I gave the name of '*capsulatum*-form'. Later, by the third or fourth week after inoculation, the morphology of the parasite is completely altered: at this stage of the induced sickness, we can observe in the tissues many large ovoid forms measuring 13–15μ in their largest diameter. This is precisely what I called the '*duboisii*-form'. When the '*duboisii*-form' appears, the '*capsulatum*-form' vanishes and we do not yet know if the invasion of the other tissues is made by the *capsulatum*- or by the *duboisii*-form.

A careful search in the medical literature and the very generous help of Dr Duncan permitted us to find eight other cases which should probably be classified as African histoplasmosis. Later, Clarke, Walker & Winston (1953) added a tenth case of Nigerian origin.

We could discuss at length the saprophytic morphology of *H. capsulatum* and of *H. duboisii*: these parasites in their saprophytic stage are not very different. But certainly they differ completely in their parasitic aspect.

The patient of Dubois, Janssens & Brutsaert (1952), on whom my mycological observations on *H. duboisii* were made, was also the starting-point of epidemiological investigations. By the use of the histoplasmin skin-test, it was possible to find among some native populations of the lower Congo as many as 10% positive reactors. So, starting from this unique case, we have discovered a new and important problem for future investigation. Classical histoplasmosis caused by *H. capsulatum* has also been found in Africa. The importance and the relation between these two diseases is a major problem for the future.

CHROMOBLASTOMYCOSIS

It was rather a surprise to discover chromoblastomycosis in the Belgian Congo. Not that there was any objection to this geographical location for this mycosis; on the contrary. But the symptomatology of chromoblastomycosis is so characteristic that we thought that any case which might have occurred should have been recognized without difficulty. They were not. When the native patients sought for relief, their cases were diagnosed as yaws, and, consequently, they were given bismuth and/or arsenic. These drugs were ineffective and that is why we found the patients. There is no medical treatment for chromoblastomycosis. Pentamidine has been given a fair trial in the Belgian Congo without success. Consequently, surgery must be resorted to as soon as possible. I would like to insist upon a small point often overlooked in the literature. It is usually quite easy to make a diagnosis of chromoblastomycosis by merely examining the scales from the verrucous lesions. In these scales, which are, actually, a mixture of epidermal cells and of pus coming from the depth, very typical fumagoid forms or sclerotic cells are to be found. In my opinion, if the clinical symptoms can be supplemented by the presence of fumagoid forms in the scales, the decision has to be surgery. Later the histological sections and cultures made on tissues removed during the operation will give the ultimate proof that the diagnosis was correct.

The second point which I would like to make is that I have classified all the isolates obtained from Congolese chromoblastomycosis as *Phialophora pedrosoi*, but I never found in the culture the form *Phialophora* which is said to appear in the colonies of *P. pedrosoi*. Nevertheless, I did not feel justified in creating a new species for the Congolese *Phialophora* because there were too many positive characters favouring the diagnosis of *P. pedrosoi* against a single negative character.

TORULOSIS

If I refer to torulosis, two cases of which have been diagnosed in the Belgian Congo, it is only to come back to an old and vexed point: the sexuality of *Torulopsis* or *Cryptococcus neoformans*. Todd & Herman (1936), and after them Redaelli, Ciferri & Giordano (1937), insisted on the fact that they observed in old cultures of *T. neoformans* some special forms which were described with full details. They concluded that the fungus had a sexual cycle. Using an isolate from one of the Congolese cases (Vanbreuseghem, 1953), I cultivated it on plaster of Paris to try and induce the production of ascospores when I observed something new to me. Some cells were very enlarged, from 2 or 3 times the volume of the normal cells. The capsule of the cell was broken at one of the poles and, through the opening, the cell itself made its way into the surrounding medium. I called these cells: 'formes d'expulsion' which can probably be translated as 'expulsive-forms'. I also observed some cells which seemed to be the result of some fusion but I would not dare to follow Todd & Herman, nor Redaelli, Ciferri & Giordano, in their conclusions on the sexual nature of this phenomenon. The reason why I recall my own observations is that in their latest book on the yeasts, Lodder & Kreger-van Rij (1952) felt justified in maintaining the genus *Cryptococcus* on a basis which neglects completely the facts observed by Todd & Herman and by Redaelli, Ciferri & Giordano.

RHINOSPORIDIOSIS

The last topic to which I would like to call attention is Rhinosporidiosis, the so-called mycosis produced by *Rhinosporidium seeberi*. And I think it is a good place to speak of this problem because it was in Great Britain that the classical paper on this condition was written, some 30 years ago, by Ashworth (1923). This disease is characterized by the formation of polyps in the nasopharynx or, but more rarely, on the ocular conjunctiva. A section made through the polypoid tissues reveals the presence of an infinity of cysts which have been described as sporangia. They are thick-walled, up to 300μ in diameter, and contain a very large number of spores. A fact often overlooked in the books is that these spores contain something. This something was not neglected by Ashworth. On the contrary, it constitutes one of the main points of his arguments. In the spore at maturation it is easy to observe, with or without staining, from ten to twenty roundish corpuscles from 2 to 3μ in diameter. These corpuscles had been called 'sporules' by some of the authors who had written on this subject before Ashworth. It was believed that the sporules were some type of small spores by which the infection was transmitted. The opinion of Ashworth was different: he believed that the 'sporules' were merely food material which was there to help the new organism in its first stage of development. Ashworth considered the matter settled: he found a single nucleus in each spore.

I had never seen a rhinosporidiosis case until lately. Our third Congolese case, which has still to be published, gave me the opportunity to make some observations which I would like to communicate.

At first, I was astonished by the regularity of the sporules; their size is quite regular, their number rather constant, and when they are out of the spore, they look like an organism and not a mere piece of 'substance de réserve'. I thought that if these sporules were not living at least they looked as if they might be. I thought also that it was easy to demonstrate, by the Feulgen stain, the presence of desoxyribonucleic acid, and consequently to locate the nuclei or nucleus in the spores. The results of my investigations are not conclusive, but they favour the interpretation of the sporules as living organisms rather than as mere reserve substances. As a matter of fact, the Feulgen stain indicates clearly that there is not something like a large nucleus in the spore, but nuclear substances attached to the sporules. When the sporules are isolated, it is sometimes possible to notice the presence of a small point of desoxyribonucleic acid attached to it.

Another point, staining by the Ziehl method indicates that the spore membrane is not acid-fast, but that the sporules are clearly acid-fast. If we accept the hypothesis that *Rhinosporidium seeberi* is a fungus, this last observation seems to be favourable to the interpretation of the sporules as spores. But I wonder; is rhinosporidiosis really a mycosis? I doubt it very much myself and feel the condition merits an unprejudiced re-examination.

It is a philosophical commonplace to say that no subject is too old to be studied anew. I feel sure there is sufficient matter in any subject to merit re-study. This seems to be specially true for medical mycology. We medical men specially interested in the mycological problems, must discipline ourselves to conform to the rules of botany, a science which seems sometimes to be more barbarian and less human than medicine. Botanists, on the other hand, whose main subject of study is medical mycology have much to learn about the somewhat riotous reactions of the human host and they must also learn to accept, from time to time, what has already been demonstrated by medical men but badly expressed. I feel sure that together we have a splendid work to accomplish in the years to come.

REFERENCES

Ashworth, J. H. (1923). On *Rhinosporidium seeberi* (Wernicke, 1903), with special references to its sporulation and affinities. *Trans. roy. Soc. Edinb.* **53**, 301–342.

Clarke, G. H. V., Walker, J. & Winston, R. M. (1953). African Histoplasmosis *J. trop. Med. (Hyg.)*, **56**, 277–280.

Dubois, A., Janssens, P. G. & Brutsaert, P. (1952). Un cas d'histoplasmose africaine. Avec une note mycologique sur *Histoplasma duboisii*, n.sp. par R. Vanbreuseghem. *Ann. Soc. belge méd. trop.* **32**, 569–584.

Duncan, J. T. (1947). A unique form of Histoplasma. *Trans. R. Soc. trop. Med. Hyg.* **40**, 364–365.

Gordon, M. A. (1951). Lipophilic yeastlike organisms associated with Tinea versicolor *J. invest. Derm.* **17**, 267–272.

Lejeune, A. (1952). Contribution à l'étude du Pityriasis versicolor au Congo Belge. *Ann. Soc. belge Méd. trop.* **32**, 521–528.

Lodder, J. & Kreger-van Rij, N. J. W. (1952). *The yeasts, a taxonomic study.* North Holland Publishing Company.

Porto, J. A. (1953). The use of cellophane tape in the diagnosis of Tinea versicolor. *J. invest. Derm.* **21**, 229.

Redaelli, P., Ciferri, R. & Giordano, A. (1947). *Boll. Sez. ital. Soc. int. Microb.* **1**, 1.

Todd, R. L. & Herman, W. W. (1936). The life-cycle of the organism causing yeast meningitis. *J. Bact.* **32**, 89–102.

Vanbreuseghem, R. (1950). Un problème de mycologie médicale: le Pityriasis versicolor. *Ann. Inst. Pasteur*, **79**, 798.

Vanbreuseghem, R. (1952*a*). Le cycle biologique des dermatophytes et l'épidémiologie des dermatophytes. *Arch. belges Derm.* **8**, 268–276.

Vanbreuseghem, R. (1952*b*). Les dermatophytes du Congo Belge. *VI. Congresso Internacional de Patologia comparada, Madrid*, pp. 4–7.

Vanbreuseghem, R. (1952*c*). *In* Langeron, M. & Vanbreuseghem, R. *Précis de Mycologie.* 2me éd. Paris: Masson.

Vanbreuseghem, R. (1953). Torulose et *Torulopsis neoformans* *Ann. Soc. belge Méd. trop.* **33**, 495–502.

Vanbreuseghem, R. (1954). Morphologie parasitaire de l'agent du Pityriasis versicolor: *Malassezia furfur* (avec la collaboration technique de P. Doupagne). *Ann. Soc. belge Méd. trop.* **34**, 251–254.

Vanbreuseghem, R. & de Tiège, R. (1952). Contribution à l'étude du Pityriasis versicolor et de *Pityrosporum ovale*. *Ann. Soc. belge Méd. trop.* **32**, 521–528.

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Trans. Brit. mycol. Soc. **38** (1), 17–30 (1955).

ON *HELICODENDRON TUBULOSUM* AND SOME SIMILAR SPECIES

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(With 10 Text-figures)

The history and literature of *Helicodendron tubulosum* (Riess) Linder is surveyed. A number of species of *Helicodendron*, which resemble more or less closely one or other of the descriptions of *H. tubulosum*, have been isolated from leaves decaying in static water. None of these isolates will produce spores under water, but spores are usually formed freely when the substrate on which the fungus is growing is exposed to a moist atmosphere in the light. These isolates are described under the following names: *H. tubulosum* (Riess) Linder, *H. conglomeratum* sp.nov., *H. articulatum* sp.nov. and *H. luteo-album* sp.nov. The very common *H. triglitziensis* (Jaap) Linder is also briefly considered.

INTRODUCTION

Riess (1853) gave the name *Helicomyces tubulosus* to a fungus growing on willow wood decaying under water at Cassel, Germany. His fungus appeared as whitish powdery tufts with spores in chains, each spore consisting of a hypha coiled 4–9 times to form a short tube; a tube of seven coils measured $35 \cdot 4 \times 17 \cdot 2\,\mu$. He figures a tightly coiled clockwise* spore of six coils measuring about $30 \times 15\,\mu$. A spore consisting of four counter-clockwise coils is also figured.

Cooke (1875) referred a fungus growing on rotting wood in Hereford to this species. He pointed out, however, that he had not seen the specimen described by Riess, and that the fungus he examined differed from the original description in having brownish spore-threads with cells about $10\,\mu$ in diameter. He figured a number of more or less conical loosely coiled, counter-clockwise spores.

Saccardo (1886) recorded *H. tubulosus* Riess as having been found at Cassel, Germany and at Hereford, England. He described the conidia as brown coiled tubes, the conidial hypha consisting of subcubical joints $10\,\mu$ in diameter. A tube of seven coils measured $35–38 \times 18\,\mu$. Probably this is a composite diagnosis derived from the descriptions of both Riess and Cooke.

Massee (1893) gave a very similar diagnosis and included a figure of a spore resembling those of Cooke, but coiling clockwise.

Zalewski (1888) described and figured coiled tubular spores, similar to those described by Riess, as one of the spore forms of *Clathrosphaera spirifera*.

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* The terms 'clockwise' and 'counter-clockwise' are defined by Davidson & Gregory (1937) as follows: 'An observer situated outside the spiral, and on its axis, describes the structure as *clockwise* if the end of the coil nearer him points in the direction of rotation of the hands of a clock. Conversely, when the end of the coil nearer him points in a direction contrary to the rotation of the hands of a clock the spiral is said to be counter-clockwise.'

It now appears that each of the spore forms described by Zalewski belongs to different species (van Beverwijk, 1951) and it is, therefore, quite likely that the coiled spore belongs to the same species as the one described by Riess.

Linder (1929) described and figured some spores found on very wet decayed twigs of *Salix lucida* at Cambridge, Mass. Later he isolated two species in pure culture which he called *Helicodendron triglitziensis* (Jaap) Linder and *H. tubulosum* (Riess) Linder. He transferred from the genus *Helicomyces* all those species which have spores coiled in three dimensions to *Helicodendron* which was proposed by Peyronel (1918) with *H. paradoxum* as the type species. *Helicodendron* differs from the somewhat similar genus *Helicoon* in the proliferation of the spores to form chains or clusters. Linder gave the following diagnosis for *Helicodendron tubulosum*: 'Colonies as shining white, scattered, minute, granular tufts of spores on the surface of the substrate. Conidiophores inconspicuous, 0–50–100 × 3·5–5 μ. Conidia acrogenous in branching chains, coiled 4–12 times to form cylindrical-ellipsoidal bodies 18–22 × 20–32 μ, the filaments 2·5–3·6 μ thick.' Examination of Linder's figures shows that most of those drawn from natural substrata have many-septate spores which are coiled clockwise, while those figured from pure cultures have counter-clockwise spores with fewer cross-walls and much less regular coils. Linder pointed out that Cooke's illustrations differ from his material in the coiling of the conidium to form a rounded cone-shaped body, deeply constricted at the septa. He could find no spores on Cooke's material at Kew. I have also examined this with the same result.

Linder (1929) described a new species, *H. hyalinum*, from herbarium material collected by Thaxter in 1899. His diagnosis is as follows: 'Colonies white, scattered, minute, visible only under the low power of the microscope. Conidiophores hyaline, ascending, sparsely branched, 15–40 × 1·3–2 μ. Conidia hyaline, the filament 1·2–1·75 μ thick, coiled in three planes to form short, 2–5 seriate, cylindrical spore bodies 11 × 8–10 μ. On decaying wood.' Some of the spores he figures are slightly larger than the limits specified and have more than five coils. I have examined Thaxter's material and, although small white patches of mycelium were visible on the surface of the wood, I was unable to find recognizable spores.

Fallen leaves of deciduous trees collected from the bottom of ponds and kept damp, but not submerged, in Petri dishes exposed to light frequently develop spores of a helicoid nature. From spore suspensions of these a number of fungi have been isolated. Several of these isolates agree more or less closely with one or other description of *H. tubulosum*, but clearly they do not all belong to the same species. Miss van Beverwijk of Baarn has also isolated fungi with helicoid spores from submerged decaying leaves and subcultures of her isolates have been examined. It seems that there are four clearly defined species involved which are described below. A short description of *H. triglitziensis* (Jaap) Linder is also included. *H. giganteum* Glen-Bott has recently been described. These six species, together with *H. hyalinum* Linder, appear to be the only known members of the genus with hyaline or subhyaline spores.

I have studied these fungi in pure culture on malt agar and on sterilized beech leaves, and there has not been any very significant difference in the spores produced on these two media. However, Miss van Beverwijk has studied isolates of some of these fungi on cherry agar and on lupin stems, and has found that the number of coils and the ultimate size of spores appear to vary not only with the age of the culture, but also with the type of medium (*in lit.*)

Helicodendron tubulosum (Riess) Linder (Figs. 1, 2)

The following revised diagnosis of this species is suggested.

Aero-aquatic fungus with branched septate mycelium. Conidium hyaline, sometimes becoming pale brown after some months, up to about 60 × 30 μ, consisting of a sparsely septate hypha 3·5–8 μ thick, coiled counter-clockwise to form a three-dimensional helix of up to fifteen coils. Material has been deposited in Herb. I.M.I. under the accession number 38967.

This fungus has been found throughout the year on a variety of different leaves collected from the bottom of ponds in a number of localities. Glistening white patches, each about the size of a pin-head, appeared on the surface of these leaves about 2 or 3 days after they had been placed in a moist atmosphere in the light. When examined under the microscope these patches proved to be made up of branched chains of helicoid spores.

This fungus has been isolated several times from beech and birch leaves collected from Chislehurst, Wisley and Waggoner's Wells. Further, three isolates have been obtained from the Centraalbureau voor Schimmelcultures, Baarn. A 5-day-old colony on malt agar, incubated at 20° C., measured about 8 mm. in diameter. In a growing colony the margin is white but the older parts grade from green to black near the centre. Most isolates sporulated fairly readily after about a week if kept in the light. In the dark the cultures either failed to produce spores or sporulation was very sparse. Isolates vary in their ability to produce spores. For instance, one Baarn isolate produced spores only after several weeks' growth, but the isolate from Waggoner's Wells sporulated all over the colony when it was about 2 weeks' old. Some isolates showed a tendency to sector into profusely sporing and sparsely sporing regions. The fungus also grows readily on sterilized beech leaves submerged in conical flasks containing sterile water. If, 2 or 3 weeks after inoculation, such leaves are placed in Petri dishes under damp conditions, spores are usually produced freely on their surface and on the surrounding film of water.

Chains of spores are formed on erect conidiophores 100 μ or more in length and 4–5 μ wide. Each conidiophore normally produces about three spores in succession side by side; subsequently each of these spores may proliferate to form branched chains (Fig. 1). The young spores seem always to be loosely coiled, but normally the coils tighten with maturity. Fully developed spores have up to fifteen coils and each is formed from a sparsely septate conidial filament 3·5–8 μ thick. Mature spores are very variable in size, but may reach about 60 × 30 μ, depending on the thickness of the filament, on the number of coils and on the tightness of coiling. A fairly tightly coiled spore of seven gyres measures about 35–40 × 22 μ. In all the spores in all the isolates I have examined, the direction of coiling has been counter-clockwise (Fig. 2). Young spores are hyaline, but they usually become pale brown about 2 or 3 months after formation. Mature spores are easily detached, and if a sporing culture is flooded with water they float to the surface where they germinate freely by tubes originating from several cells of each spore. This behaviour is to be found in all the species of *Helicodendron* that I have studied.

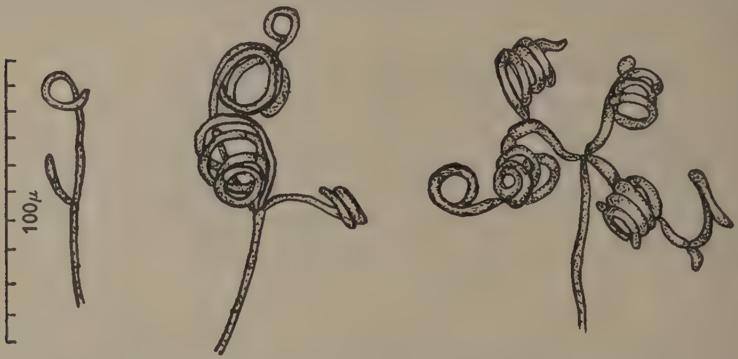

Fig. 1. *H. tubulosum* (Riess) Linder. Spore clusters at various stages of development (from pure culture on malt agar).

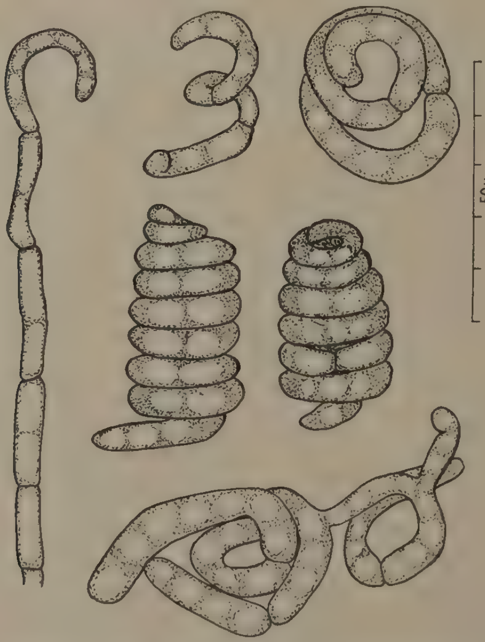

Fig. 2. *H. tubulosum* (Riess) Linder. Spores at various stages of development (from pure culture on malt agar).

Judging from Linder's figures (his pl. 27, figs. 7, 11) of counter-clockwise spores, this fungus is the most like his *H. tubulosum* of any of the isolates. The largest spore he figures has only six coils and measures only 30 × 20 μ.

Although most of the spores I have observed have been larger than those recorded by Linder, I think that the difference in size can be accounted for by differences in the age of the cultures or in the conditions under which they were grown. This fungus agrees as closely as any of the isolates with the original description of Riess. It also agrees quite well with the helicoid spores described by Zalewski as a spore stage of *Clathrosphaera spirifera*. It is almost certainly not the same as the fungus described by Cooke.

Helicodendron conglomeratum sp.nov. (Figs. 3, 4)

Fungus aero-aquaticus mycelio repente ramoso septato. Conidium filiforme, multiseptatum in tubulum ovatum (circ. 30–50 × 20–30μ) vicibus 8–14 dextrorsum convolutum, hyalinum vel sub-brunneum. Conidiophora ferens glomus densum conidiorum. Culturae in agaro ('malt agar') diverse pigmentatae sed nunquam brunneae. *Hab.* In foliis putrescentibus submersis *Fagi* et *Quercus*, Windermere et Waggoner's Wells (Hindhead) Anglia. Typus: Herb. I.M.I. 38969 (Waggoner's Wells, December 1949).

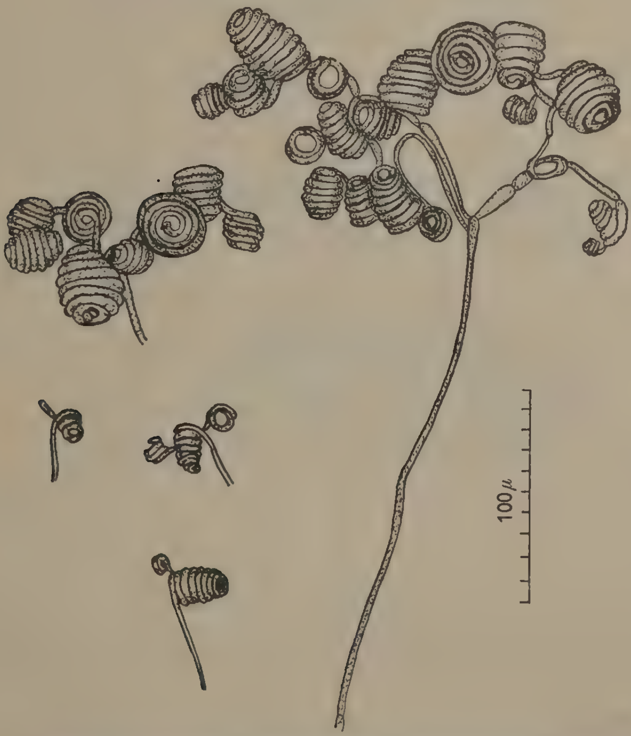

Fig. 3. *H. conglomeratum* sp.nov. Spore clusters at various stages of development (from pure culture on malt agar).

Aero-aquatic fungus with branched, septate mycelium. Conidium hyaline to pale brown, about 30–50 × 20–30μ consisting of a many-septate hypha coiled clockwise to form a three-dimensional helix of about eight to fourteen coils. Conidiophore bearing a dense cluster of spores. Colony on agar (2 % malt agar) variously pigmented but never brown.

On submerged decaying leaves of beech, oak, etc.; Windermere, Waggoner's Wells (Hindhead), England and Baarn, Holland.

Note. Differs from *H. tubulosum* in direction of coiling of spore and from *H. giganteum* in the smaller spore size.

This fungus also has been found throughout the year on a variety of different leaves collected from the bottom of ponds in a number of localities. Glistening white patches of coiled spores, indistinguishable with the naked eye from those of *H. tubulosum*, appeared on the surface of these leaves 2 or 3 days after they had been placed in a moist atmosphere in the light.

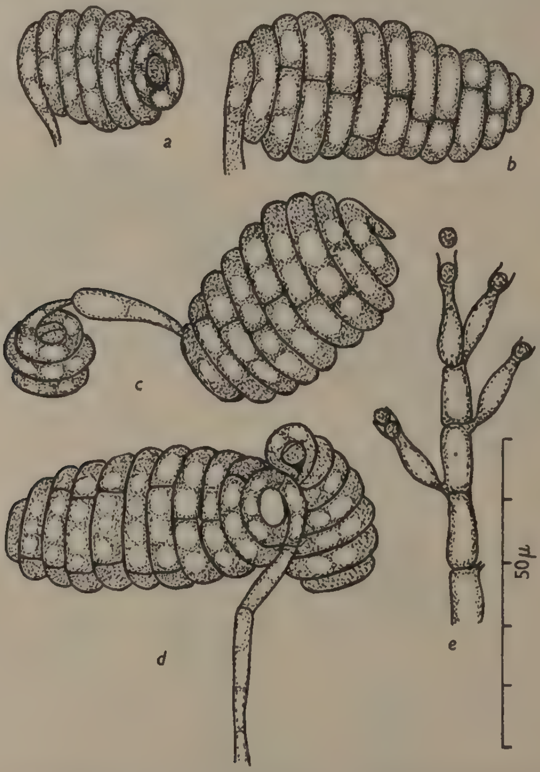

Fig. 4. *H. conglomeratum* sp.nov. *a–d*, spores at various stages of development; *e*, phialospores (from pure culture on malt agar).

Isolations in pure culture have been obtained from beech and oak leaves collected from Windermere and from Waggoner's Wells. Two isolates of the same species have been obtained from the Centraalbureau; both were from an oak twig found in a pond near Baarn. The rate of growth on 2 % malt agar is much the same as in *H. tubulosum*; a 5-day old colony incubated at 20° C. measured 7 mm. in diameter. The general appearance of the colony is much like that of *H. tubulosum*, but it tends to be less pigmented. The rate of pigment production varies very much in different isolates and also in different sectors of a colony. Some isolates show concentric dark and light zones. On exposure to light cultures usually develop spores fairly freely, especially on the older parts. Different isolates vary considerably in the degree of sporulation, and some tend to develop profusely sporing and sparsely sporing sectors.

The spores are produced in dense clusters at the tips of simple or sparingly branched conidiophores (Fig. 3). Observations on spores at different stages of development indicate that the conidial filament is at first a small tightly coiled helix formed at the apex of the conidiophore. Subsequent enlargement of this is by increase in thickness and also by its continued apical growth. At a fairly early stage in its development the spore usually starts to proliferate, giving rise to clusters which are distinctly more crowded than those of *H. tubulosum*. Each mature spore is composed of a many-septate filament, 3–5 μ thick, coiled tightly to form a three-dimensional helix of eight to fourteen gyres measuring 30–50 × 20–30 μ. The individual cells of the spore-filament in mature spores are slightly constricted at the cross-walls. The direction of coiling in all the spores examined has been clockwise (Fig. 4). Spores are hyaline at first, but in cultures kept for 2 or 3 months they may become pale brown. Two isolates of this fungus have also been observed to form phialospores (Fig. 4), but these have not so far been seen to germinate.

This fungus shows a very marked resemblance to *H. giganteum*, both in its method of spore formation and in the type of colony produced. The significant differences between the two species are first that the upper limit of spore size is not as great as in *H. giganteum*, secondly proliferation of the spores is more extensive.

It seems likely that the clockwise spores drawn by Linder from naturally occurring substrates (his pl. 27, figs. 9, 10) and ascribed by him to *H. tubulosum* really belong to *H. conglomeratum*.

Helicodendron articulatum sp.nov. (Figs. 5, 6)

Fungus aero-aquaticus mycelio repente ramoso, septato, 5–7 μ dia. Conidium subbrunneum, in hypha multiseptata consistens ad septum quodque constricta et vicibus 5–12 dextrorsum laxe convoluta. Culturae in agaro ('malt agar') sub-brunneae. *Hab.* In folio putrescenti submerso *Fagi*, Waggoner's Wells (Hindhead), Anglia, Martins, 1949. Typus: Herb. I.M.I. 48341.

Aero-aquatic fungus with branched septate mycelium 5–7 μ thick. Conidium light brown, consisting of a many-septate hypha, constricted at the septa and coiled loosely in a clockwise direction to form a helix of about five to twelve coils. Colony on agar (2 % malt agar) pale brown. On a submerged decaying leaf of beech; Waggoner's Wells (Hindhead), England.

This fungus has been found on one occasion only, on a submerged decaying beech leaf collected from Waggoner's Wells in March 1949. Clusters of loosely coiled pale brown helicoid spores appeared on the surface of the leaf after it had been kept damp in a Petri dish for a few days.

A 5-day culture on malt agar incubated at 20° C. had a diameter of about 19 mm. Thus the rate of extension is double that of either of the two preceding species. The colony is at first flat and pale yellow in colour, never black. Subsequently, it usually becomes covered with loose aerial mycelium, and later with pale brown spores. Exposure to light was not necessary for sporulation.

The spores, which proliferate to form clusters, are formed terminally and laterally on simple or sparingly branched conidiophores 3–5 μ thick (Fig. 5). Each mature spore is loosely coiled to form a three-dimensional helix of about five to twelve gyres; a spore of seven gyres measures about 40 × 28 μ. The conidial filament is markedly constricted at the cross-walls which are 10–15 μ apart. The direction of coiling appears to be constant and clockwise. Observations on spores at different stages of development indicate that they originate from loosely coiled septate hyphae about 3 μ thick; enlargement of the spore being brought about subsequently by increase in thickness of the conidial filament up to about 7 μ and also by its continued apical growth (Fig. 6). The spores of this fungus resemble very closely those figured by Cooke, but coiling is in the opposite direction.

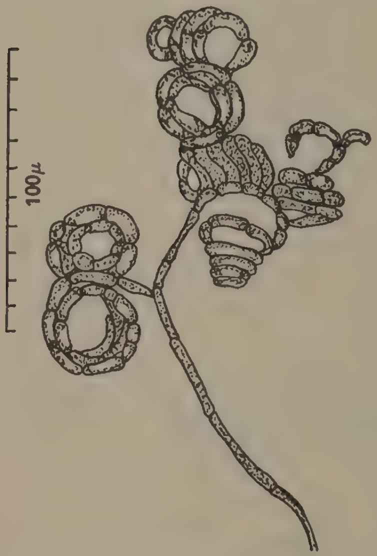

Fig. 5. *H. articulatum* sp.nov. Spore cluster (from pure culture on malt agar).

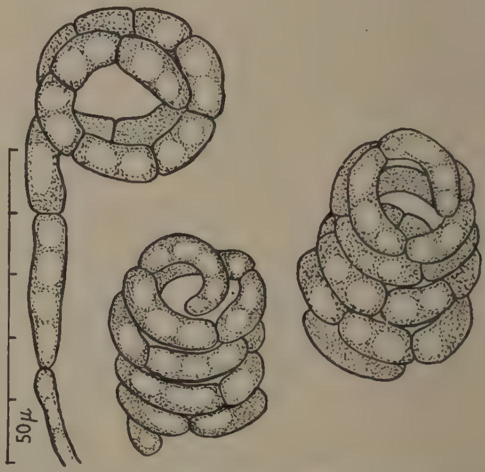

Fig. 6. *H. articulatum* sp.nov. Spores at various stages of development (from pure culture on malt agar).

Helicodendron luteo-album sp.nov. (Figs. 7, 8)

Fungus aero-aquaticus mycelio repente ramoso, septato. Conidium hyalinum vel luteo-album, in hypha multiseptata consistens, 1·5–2 μ dia., 4–16 vicibus sinistrorsum artius laxius convoluta. Culturae in agaro ('malt agar') albae vel luteae, raro cineraceae, nunquam nigrae. *Hab.* In foliis putrescentibus submersis *Quercus* et *Fagi*, Waggoner's Wells (Hindhead) Anglia et in folio *Salicis*, Swanley, Kent, Anglia. Typus: Herb. I.M.I. 38966, *Quercus*, Waggoner's Wells, December 1949.

Aeroquatic fungus with branched septate mycelium. Conidium hyaline or very pale yellow, consisting of a many-septate hypha, 1·5–2 μ thick, coiled more or less tightly in a counter-clockwise direction to form a three-dimensional helix of four to sixteen coils. Colony on agar (2 % malt agar) white to yellow, rarely grey, never black.

This fungus has been isolated from submerged oak and beech leaves collected at Waggoner's Wells, Surrey, and from a willow leaf from a pond near Swanley, Kent. Spores were produced when the leaves were kept under damp conditions. A 5-day-old colony on malt agar incubated at 20° C. measured 6 mm. in diameter. The mycelium is at first white and mainly submerged or at the surface; later aerial hyphae develop and the colony usually becomes pale yellow. All the original isolates sectored freely, often causing the colony to become crenated, and they all produced a certain amount of orange pigment in irregular patches in the older parts of the colony; in one isolation grey pigment was produced in some sectors. On exposure to light, sporulation usually occurred over the whole surface of the colony.

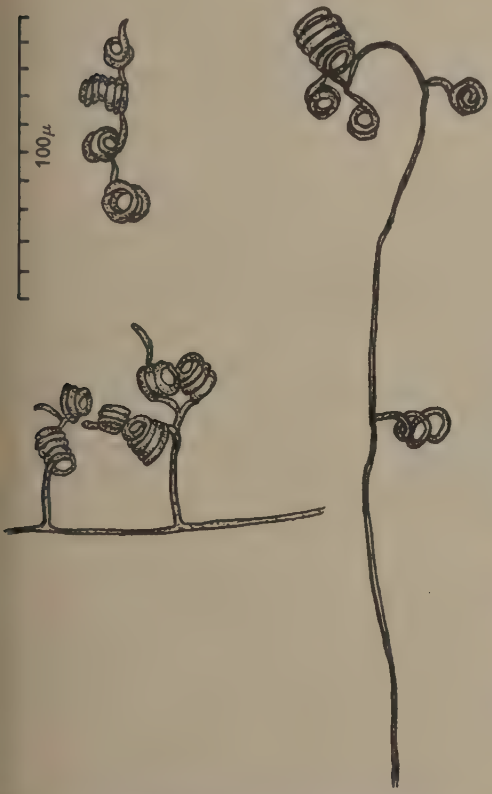

Fig. 7. *H. luteo-album* sp.nov. Spore clusters at various stages of development (from pure culture on malt agar).

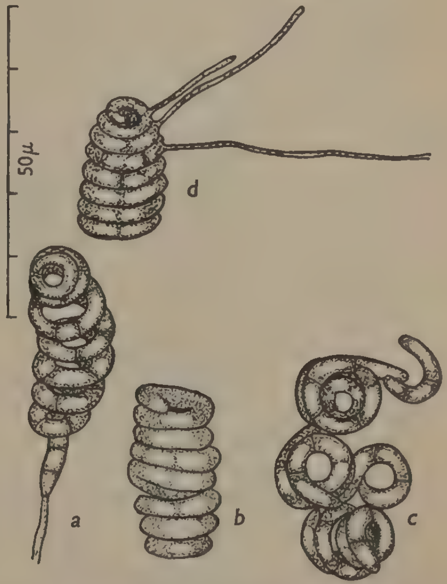

Fig. 8. *H. luteo-album* sp.nov. *a–c*, spores at various stages of development (from pure culture on malt agar); *d*, germinating spore (from pure culture on beech leaves).

Branched chains of spores are produced terminally and laterally on simple or sparingly branched conidiophores 1·5–2 μ in diameter and of variable length up to about 300 μ (Fig. 7). Each spore is formed by the apical growth of a conidial filament 2–3 μ thick, to form a three-dimensional helix of four to sixteen gyres. The spores vary in size, depending on the number of coils and the tightness of coiling. A tightly coiled spore of seven gyres measures about 20 × 12 μ. The spores are many-celled, but not constricted at the cross-walls. Mature spores are hyaline to very pale yellow. In all the isolates examined the spores were coiled in a counter-clockwise direction (Fig. 8).

The spores of this fungus are intermediate in size between those of *H. tubulosum* and *H. hyalinum*. It is, however, clearly not the same species as the isolates attributed to *H. tubulosum*, and it differs from *H. hyalinum* in the larger size of its spores and in their colour.

Helicodendron triglitziensis (Jaap) Linder (Figs. 9, 10)

This fungus seems to be the commonest species of *Helicodendron*; it has been found in practically every sample of leaves and twigs collected from the bottom of stagnant ponds. It has also been found fairly frequently on leaves collected from the surface of the water. White glistening clusters of spores appeared on the surface of the leaves after they had been kept for a few days in light under damp conditions.

Isolations have been obtained from leaves collected from ponds at Chislehurst, Guildford, and Swanley. Growth and sporulation of these isolates on malt agar and on sterilized beech leaves have been studied and compared with that of an isolate from a birch leaf from a pond near Omen, Holland, sent from the Centraalbureau. This species grows rapidly on malt agar; a colony grown at 20° C. for 5 days has a diameter of about 35 mm. Much white surface, and later aerial, mycelium is formed and, after being kept in the light for a few days, spores usually appear over the whole surface of the colony. Occasionally, instead of coils, chains of moniliform spores are produced. Cultures kept entirely in the dark have never been observed to sporulate. Young cultures are not coloured, but when the colonies are several months old the mycelium and sometimes the spores become pale brown. Several isolates have been observed to sector into freely sporing, sparsely sporing, and non-sporing regions.

Branching chains of loosely coiled hyaline spores are produced both terminally and laterally on short erect conidiophores which are simple or sparingly branched (Fig. 9). Each mature spore consists of a conidial filament 3–5 μ thick, usually of three to nine cells, and coiled loosely 1–1½ times to form a three-dimensional helix about 25 μ in diameter. Observations on spores at various stages of development indicate that the first spore of a series is normally formed by the apical growth of the conidiophore, and when more or less fully grown the coiled spore becomes delimited from the conidiophore by a cross-wall. At this stage the spore usually consists of two or three cells, any of which may develop lateral buds which grow out to produce more helicoid spores which become constricted from the parent at their points of origin. These daughter spores may proliferate in the same way, giving rise to long-branched chains of spores which eventually become a tangled mass. This may become detached and dis-

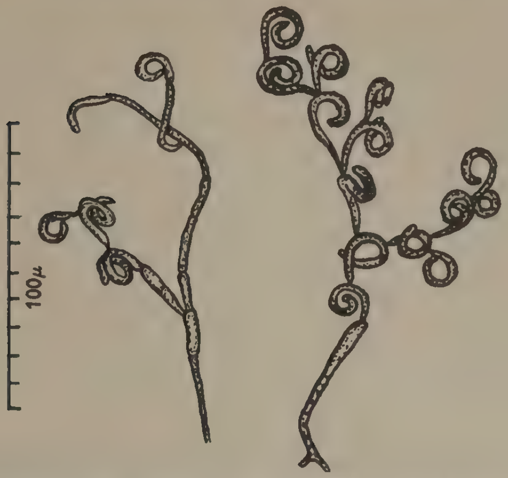

Fig. 9. *H. triglitziensis* (Jaap) Linder. Spore clusters at various stages of development (from pure culture on malt agar).

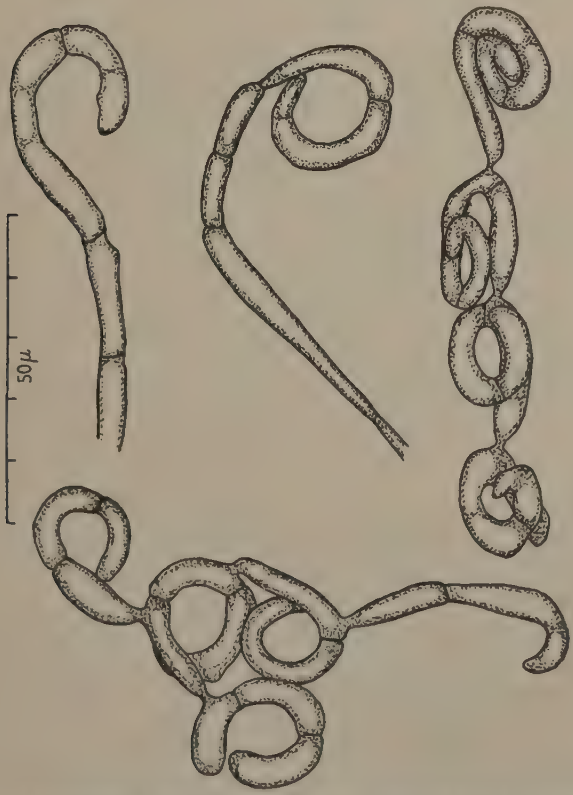

Fig. 10. *H. triglitziensis* (Jaap) Linder. Spores at various stages of development (from pure culture on malt agar).

persed as a single unit, although if such a unit is mounted in water under a cover-slip it then breaks up readily into the individual coils. The direction of coiling is not constant, clockwise and counter-clockwise coils being found in the same cluster (Fig. 10).

All the isolates examined agree very well with Linder's figures and description of *H. triglitziensis*. It is interesting to note that Linder first noticed spores of this fungus developed just above the water-line from twigs of *Salix lucida* which had been collected from a small pond and kept in water. Linder investigated the sporulation of this fungus in pure culture and found that exposure to sunlight was a necessary condition; he also states that spores were produced only after the material had been exposed to freezing conditions and then returned to the temperature of the laboratory. He suggests that this explained the occurrence of the fungus only in October and November, during which a relatively low night temperature is followed by a higher day temperature. However, I have obtained spores of this fungus from leaves collected in late May, early September and January. Also, isolates from leaves collected in September, 1948, still sporulate freely on subculturing without any special low-temperature treatment, so long as the culture is exposed to sunlight.

Discussion

The species described above form part of the fungal flora of fallen leaves of deciduous trees decaying submerged in more or less static water. Most of these fungi produce spores only when the leaves become exposed to the air or when the water is so shallow that hyphae can grow up to the surface and produce spores there. Van Beverwijk (1951) has used the term 'aero-aquatic' fungi for these organisms.

A large proportion of these aero-aquatics belong to the genus *Helicodendron*, and an attempt has been made in this paper to sort out the various species which resemble more or less closely the original description of *Helicomyces tubulosus* Riess. Other species of *Helicodendron* with hyaline or sub-hyaline spores have also been considered.

The main criteria on which I have based the division of these fungi into species are as follows: (1) direction of coiling of spore; (2) thickness and septation of conidial filament; (3) looseness or tightness of coiling; (4) number of coils and approximate ultimate size of spore; (5) cultural characters.

In some species (*H. triglitziensis*, and the dark-spored *H. paradoxum*) the direction of coiling of the spores is not constant, clockwise and counter-clockwise spores being found on the one colony, but in most of the species encountered the direction of coiling has been constant not only in each individual isolate, but for every isolate of the one species. However, in no case have two species been delimited solely on the direction of coiling of the spores, but if a detailed investigation of the growth of these fungi in culture is not possible, the direction of coiling does serve to distinguish between species such as *H. tubulosum* and *H. conglomeratum* in which the mature spores are very much alike.

The thickness and septation of the conidial filament is sometimes a fairly constant feature, as in *H. articulatum* with its many-septate hypha 5–7 μ thick which is markedly constricted at the cross-walls, and in *H. luteo-album* in which the many-septate filament is only 2–3 μ thick and is scarcely constricted at all. In *H. tubulosum*, however, the thickness of the conidial filament varies from 3·5 to 8 μ.

Looseness or tightness of coiling is also a rather variable feature in some species (*H. tubulosum* and *H. luteo-album*) but *H. conglomeratum* and *H. giganteum* have spores which are consistently tightly coiled, while the spores of *H. articulatum* are consistently loosely coiled.

The number of coils, and the ultimate size of the spore is an extremely difficult feature to define exactly in any of these species. It seems that in most species spores may become detached from their conidiophores and germinate while they consist of only a few coils; if, however, they do not become detached they frequently continue to increase in size and in the number of their coils by the apical growth of the conidial filament. Only in two species examined (*H. triglitziensis* and *H. conglomeratum*) is the number of coils in mature spores fairly constant.

The following is a key for the identification of the hyaline and subhyaline species of *Helicodendron*.

Key to the hyaline and subhyaline species of Helicodendron

1. Spores of less than four coils; direction of coiling variable; colony on 2% malt agar white or light brown with rapid growth (diameter of colony more than 30 mm. in 5 days at 20° C.). **H. triglitziensis**

1. Spores of more than four coils; direction of coiling constant; slow growth on 2% malt agar (diameter of colony less than 20 mm. in 5 days at 20° C.). 2

  2. Direction of coiling counter-clockwise 3
  2. Direction of coiling clockwise 4

3. Conidial filament less than 3 μ thick; colony on malt agar never black. **H. luteo-album**

3. Conidial filament more than 3 μ thick; colony on malt agar usually becoming black. **H. tubulosum**

  4. Spores always light brown; conidial filament loosely coiled and markedly constricted at the cross-walls. Colony on 2% malt agar never black; diameter more than 10 mm. in 5 days at 20° C. **H. articulatum**
  4. Spores hyaline at first; conidial filament tightly coiled and only slightly constricted at the cross-walls; colony on 2% malt agar sometimes black; diameter less than 10 mm. in 5 days at 20° C. 5

5. Spores of about 8–14 coils and up to $50 \times 30\,\mu$. **H. conglomeratum**

5. Spores of up to 20 coils and $200 \times 70\,\mu$. **H. giganteum**

(*H. hyalinum* has not been included in this key, because its behaviour in culture, and the direction of coiling of its spores are not yet known.)

I wish to express my thanks to Dr Hilda Lund who collected some of the leaves from which these fungi were isolated, to Miss A. L. van Beverwijk at Baarn for many helpful suggestions and for her isolates of *H. tubulosum*, to Prof. D. M. Jones for the Latin diagnosis of the new species, to the late Prof. W. Lawrence White of the Farlow Herbarium, Harvard University, for the loan of the herbarium material of *H. hyalinum*, and especially to Prof. C. T. Ingold under whose direction this investigation was carried out.

REFERENCES

COOKE, M. C. (1875). *Helicomyces tubulosus* Riess. *Grevillea*, **3**, 178.

DAVIDSON, A. M. & GREGORY, P. H. (1937). The spiral hyphae of *Trichophyton*. *Trans. Brit. mycol. Soc.* **21**, 98–113.

ENGLER, A. & PRANTL, K. (1900). *Die natürlichen Pflanzenfamilien*, T. **1**, Abt. 1**, Leipzig.

GLENN-BOTT, J. I. (1951). *Helicodendron giganteum* n.sp. and other aerial sporing hyphomycetes of submerged dead leaves. *Trans. Brit. mycol. Soc.* **34**, 275–279.

JAAP, O. (1916). *Helicomyces triglitziensis*. *Verh. bot. Ver. Brandenb.* **58**, 43.

LINDER, D. H. (1925). Life history of *Helicodesmus albus*. *Amer. J. Bot.* **12**, 259–269.

LINDER, D. H. (1929). Helicosporous Fungi Imperfecti. *Ann. Mo. bot. Gdn*, **16**, 227–388.

MASSEE, G. (1893). *British fungus flora*, **3**. London.

PEYRONEL, B. (1918). *Helicodendron paradoxum*. *Nuovo G. bot. ital.*, N.S., **25**, 460.

RIESS, H. (1853). *Helicomyces tubulosus*. *Bot. Ztg*, **11**, 140.

SACCARDO, P. A. (1886). *Sylloge Fungorum*, **4**. Patavii.

VAN BEVERWIJK, A. L. (1951). Zalewski's '*Clathrosphaera spirifera*'. *Trans. Brit. mycol. Soc.* **34**, 280–290.

ZALEWSKI, A. (1888). Przyczynki do życioznawstwa grzybów. *Rozpr. Akad. Um. (mat.-przyr.)*, **18**, 153–199.

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Trans. Brit. mycol. Soc. **38** (1), 31–46 (1955).

PYTHIUM THALASSIUM SP.NOV. INFECTING THE EGG-MASS OF THE PEA-CRAB, *PINNOTHERES PISUM*

BY D. ATKINS
Marine Biological Laboratory, Plymouth

(With 9 Text-figures)

A marine *Pythium*, *P. thalassium* sp.nov., has been found parasitic and saprophytic in the eggs of the pea-crab, *Pinnotheres pisum*, and of other Crustacea. The sporangia are filamentous, the method of asexual reproduction is that characteristic of the genus *Pythium*: proliferation occurs. Some variation in the behaviour of the contents of the sporangium was noted. Asexual bodies, similar to those described in certain other species of *Pythium*, are borne extramatrically at the apex of hyphae. Various stages in the germination of spores were seen. Sexual organs were not present in the material examined. The affinities of *P. thalassium* are discussed.

Pythium thalassium sp.nov. (Figs. 1–9).

Mycelio plerumque intramatricali, formato de hyphis profusissime ramosis, latis 5–20 μ; sporangiis filamentosis, saepe proliferosis; vesiculis latis 70–90 μ, evanescentibus; zoosporis numerosis, longis circa 15 μ, cum duobus flagellis in latere insertis; sporis tum sphaeralibus vel ovalibus, 10·5–12 μ longis, cum in capsulas inclusa sunt; corporibus nunc sphaeralibus nunc pyriformibus, adiunctis extra matricem ad apices hypharum, germinantibus per hyphas productas; generatione sexuali ignota.

Mari incolens, parasiticum et saprophyticum in ovis *Pinnotheres pisum*, Anglia.

Mycelium mostly intramatrical, although extramatrical growth occasionally reaches 1 mm. in length. Hyphae 5–20 μ in diameter. Intramatrical mycelium richly branched. Sporangia filamentous, similar to vegetative hyphae; frequently proliferous. Vesicles extramatrical, 70–90 μ in diameter, evanescent. Zoospores numerous, grooved, laterally biflagellate, about 15 μ long; when encysted 10·5–12·0 μ in diameter, round to oval. Spherical and pyriform structures, 25–52 μ in diameter, borne extramatrically at apex of hyphae, germinate by sending out hyphae. Sexual reproduction so far unknown.

Parasitic and saprophytic in the eggs of the marine pea-crab (*Pinnotheres pisum*); Camel Estuary, Padstow, north Cornwall, June 1929.

Type material has been deposited in the British Museum (Natural History).

INTRODUCTION

During work on *Pinnotheres* at the laboratory of the Marine Biological Association, Plymouth, a *Pythium* was found in the egg-mass of a few *Pinnotheres pisum* in June 1929. *P. pisum* is a parasite of the mussel, *Mytilus edulis*, and those containing the infected crabs came from the Camel Estuary, Padstow, north Cornwall. The crabs were kept in tank water, the salinity of which was about 36–37 ‰ and somewhat higher than that of outside sea water. In water of this salinity the fungus produced and liberated swarms of zoospores in the manner characteristic of the genus *Pythium*.

In June 1953 during a return visit to Plymouth, and after this paper had been written, a single egg infected with the same *Pythium* was found among eggs cut from a dead *Pinnotheres pisum*, which had lived in the laboratory for nearly 10 months.

It is proposed to name this *Pythium*, *P. thalassium*. It belongs to the section of this genus with filamentous sporangia in no way differing from vegetative hyphae, as does also the only other marine *Pythium* so far described, *P. marinum* Sparrow, saprophytic in the red alga, *Ceramium rubrum*, collected in 10 m. of water of salinity 32 ‰ off the island of Læsö, Denmark (Sparrow, 1934).

The bodies of living crabs have not been found infected with the fungus, and although the six crabs carrying infected eggs were kept together in a bowl for 8 days they did not become infected at the end of that—perhaps short—period. But the fact that, unlike the saprolegniacean fungus (*Leptolegnia marina*) of *Pinnotheres* (Atkins, 1929, 1954), *Pythium thalassium* has so far been observed to attack only the eggs and not the living parent, would seem to indicate that the germ tube has a limited power of penetration. *Leptolegnia marina* was found to enter the crab in regions where the integument is exceedingly thin—namely, the roof of the gill chamber, and the junctions between the segments of the abdomen and between the carapace and the abdomen—but not as thin as are the egg membranes. Possibly the germ tube of *Pythium thalassium* is only able to penetrate a cuticle of the thickness of that covering the egg. It appears that, in a number of fungi, including *P. de Baryanum*, attacking plants, the mechanism of the act of penetration is mechanical and that different fungi have different penetrative capacities (Brown, 1948, pp. 174–5).

P. thalassium is a parasite of crustacean eggs and also a saprophyte. From the single infected egg found in 1953 other *Pinnotheres* eggs were infected and then the eggs of *Crangon vulgaris*, *Leander serratus*, *Portunus depurator* and *Macropodia* sp. Infection of eighteen *Macropodia* eggs followed within 2 days of placing a single infected *Pinnotheres* egg with them. Germ tubes were present within eggs containing embryos with heart beating, thus there can be no doubt but that *Pythium thalassium* is a true parasite. That germ tubes from spores lying on the surface will enter eggs with yolk degenerating and liquefied is also certain and the fungus has been encountered in egg envelopes from which the zoeae had hatched: here the mycelium was small.

Among species of *Pythium* with filamentous sporangia the only known parasite of an animal is *P. daphnidarum*, a destructive parasite of the small Cladoceran crustaceans, *Daphnia hyalina* and *D. cucullata*, as well as of *Bosmina coregoni* in lakes in Denmark (Petersen, 1910).

Lagenidium callinectes (Lagenidiales), with asexual reproduction somewhat resembling that of *Pythium*, has been described as a parasite of the egg-mass of the blue crab (*Callinectes sapidus*) from off the eastern sea board of America by Couch (1942), Newcombe & Rogers (1947) and Rogers-Talbert (1948).

DESCRIPTION OF MATERIAL

Six *Pinnotheres pisum* among 192 of both sexes taken from *Mytilus edulis* on 6 June 1929 were found to be carrying eggs infected with a *Pythium*. Incidentally, in addition to those so infected, eight females or their eggs were attacked by *Leptolegnia marina* (Atkins, 1929, 1954). The crabs carrying *Pythium*-infected eggs were recovered on the day of their arrival. Eight days later the pleopods, with attached ova, were removed and kept in sea water until 17 June when a sketch of the vesicles (Fig. 1 A) was made. From 6 to 17 June the temperature of laboratory sea water ranged from 13 to 14° C.

Notes made when the crabs were found record that nearly all eggs of the egg-mass of five were dead and infected with the fungus, while the sixth had some infected eggs.

Owing to the pressure of other research little time could be spent studying the fungus when discovered, and most of the description is based on material preserved in 5 % formalin, but the following observations were made on living material. Hyphae penetrating to the exterior of the *Pinnotheres* ovum bore terminally very thin-walled spherical vesicles, 70–90 μ in diameter (Fig. 1 A). Following rupture and disintegration of the wall of mature vesicles, grooved, laterally biflagellate zoospores were liberated and scattered in all directions. In the 1953 material the wall of vesicles seemed to be tougher and the spores tended to follow each other through one rent in the membrane, which persisted for about a minute. Variation in toughness of the vesicle membrane has been noted in *Pythium undulatum* by Goldie-Smith (1952, p. 278).

On the infected *Pinnotheres* eggs examined in 1929 the emission tubes were short, up to 60 μ in length and 10–16 μ wide (Fig. 1 A). After the vesicle had burst and the membrane disintegrated, these empty tubes were left with flared lips (Fig. 1 A, *b*).

It was evident from examination of the 1929 preserved material that the infection had not yet reached its maximum, although the *Pythium* had existed for 11 days in the laboratory. In spite of this many *Pinnotheres* eggs were coated with germinating spores. The mycelium was mostly intramatrical, the extramatrical growth consisting chiefly of emission tubes. These tubes may secondarily become sporangia, or part of sporangia, through renewed growth. In 1953 some crustacean eggs, kept at a constant temperature of 15–16° C., developed a fine cloud of radiating hyphae up to 500 μ long; small dishes containing the growing fungus had the surface film thick with spores.

The yolky eggs carried by *P. pisum* being opaque, it was only possible to see young internal growth of the fungus clearly in ova of which most of the yolk had already been liquefied and absorbed by other organisms, chiefly bacteria, before attack by the fungus. In degenerating ova with yolk reduced to a small central body, hyphae were to be seen extending from it toward the periphery. In such young mycelia all hyphae were crowded with refringent bodies, probably fat (Fig. 2 C). A somewhat older mycelium with certain of the inner hyphae containing refringent bodies and the outer with hyaline to finely granular protoplasm is shown in Fig. 2 A: it was entirely internal.

The mycelium within the host was richly branched, there being a tendency to right-angled branching (Fig. 2B), and occasionally some appearance of dichotomy (Fig. 2C). Young hyphae were without septa, but septa were not uncommon in old mycelia and where asexual reproduction was taking place.

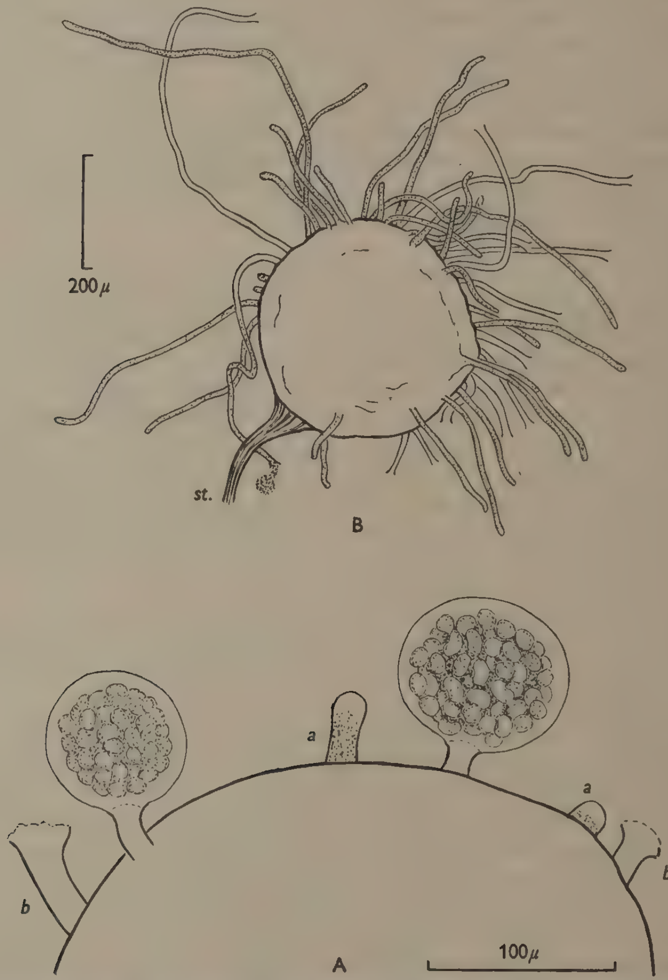

Fig. 1. *Pythium thalassium.* A: emission tubes projecting from ovum of *Pinnotheres pisum*: two bear vesicles, one with immature and the other with mature zoospores ready for liberation. Hypha, *a*, previous to formation of vesicle; *b*, empty after functioning as exit tube. B, considerable unbranched growth of fungus on *Pinnotheres* egg, intramatrical growth invisible owing to opaqueness of ovum. *st*, stalk of ovum. A, from living material; B, unstained, in 5 % formalin.

The hyphae of *Pythium thalassium* vary in width from 5 to 20μ. The general habit of growth is thus somewhat coarser than that usual in most known species of the genus. Matthews (1931, p. 43), however, reported that in *P. papillatum* the hyphae are very irregular in size, often with blunt tips, and that the main hyphae are up to 24 μ in diameter at the base, while the ordinary hyphae are about 7·2 μ thick.

External hyphae of *P. thalassium* varied in length from 26 to 1000 μ. What determines the length of external growth is unknown. Short evacuation tubes did not appear to characterize early stages of asexual reproduction, for on some ova all tubes were long. But where a few infected ova occurred among healthy ones on pleopods, suggesting that living ova had been attacked, the external emission tubes were short. In such instances the infected ova were still opaque with yolk and the internal mycelium invisible. Where most of the eggs on a pleopod were dead and degenerate, although not all infected with the fungus, and where fouling of their surface was probable, then there was frequently extramatrical growth of some length, either of unbranched evacuation tubes (Fig. 1 B) or of irregularly branched sporangia, produced chiefly by proliferation (Fig. 5). Probably when ova become much contaminated by other organisms the evacuation tubes are long so as to allow of liberation of the zoospores beyond the bacterial zone. In *P. gracile* apparently greater extramatrical growth occurred in saprophytic than in parasitic forms of the species, for Butler (1907, pp. 67–8) mentioned that in the former the mycelium grows out as a fine cloud surrounding the substratum while in parasitic forms the sporangia open to the exterior by nearly straight, rather short branches.

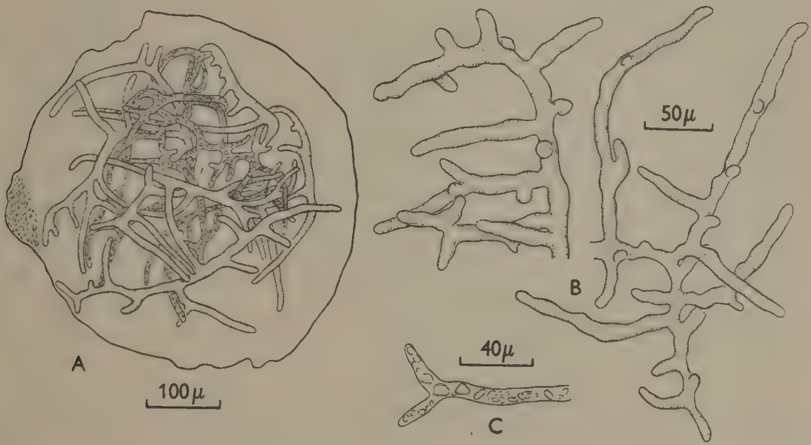

Fig. 2. *Pythium thalassium.*. A: mycelium or mycelia within an egg of *Pinnotheres pisum*, compressed by cover-slip; central hyphae crowded with somewhat irregular-shaped refringent bodies. The egg, in which little yolk remained, was coated with bacterial filaments (not shown) and a heavy growth of rod bacteria was present near the margin as indicated. B: portions of mycelia to show the method of branching. C: hypha, from a young mycelium, containing irregular-shaped refringent bodies. This, and all following figures, are from unstained material in 5% formalin.

A tendency to spiral coiling of external hyphae was exhibited by some plants (Figs. 3 A, B, 8 A). Spiral coiling has been noted in other species of *Pythium*, for instance in *P. diacarpum* (with spherical sporangia)

(Butler, 1907, p. 80) and *P. inflatum* (with inflated filamentous sporangia) (Matthews, 1931, p. 45).

Slight torulose structure in an emission tube and bud-like swellings of the external portion of a sporangium are shown in Fig. 3C, D. A peculiarity of certain plants was the presence of curiously shaped tips to the extramatrical hyphae.

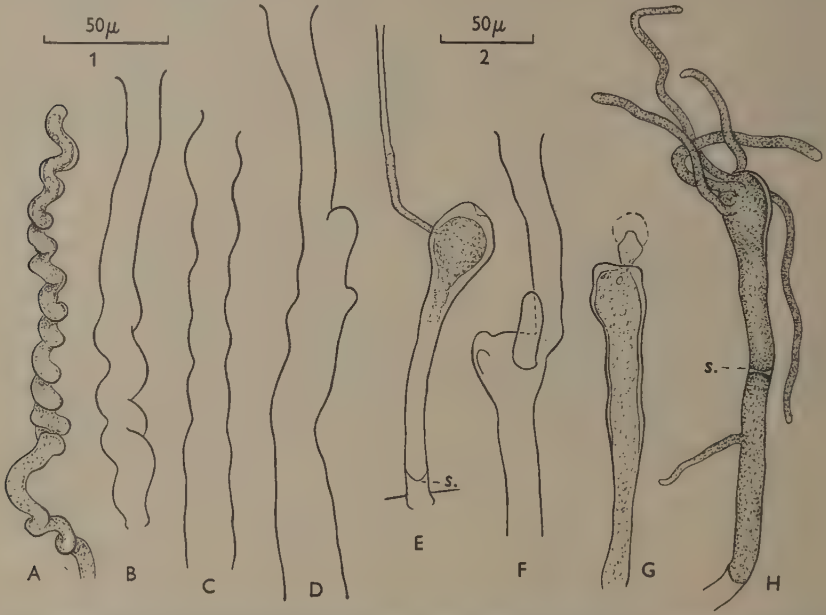

Fig. 3. *Pythium thalassium.* Extramatrical growth, some of it empty after production of zoospores. A, B, spiral growth; C, toruloid structure; D, bud-like lateral swellings; E–H, portions of sporangia with swollen ends. H shows two contiguous sporangia, the terminal one with swollen end from which five hyphae have arisen, the proximal one with a single lateral tube. *s*, septum. A–D, F, G to scale 1; E, H to scale 2.

Asexual reproduction

The filamentous sporangia may be single or branched, intramatrical or extramatrical, although generally the former in the material examined.

No pronounced enlargement of the evacuation hyphae before piercing of the egg membrane was evident; they were narrow at the point of entrance and then swelled out again. The passage of protoplasm into the vesicle and formation of zoospores have been described and illustrated by Butler (1907, pp. 26–30), Sparrow (1931, pp. 260–2) and Goldie-Smith (1952, pp. 276–8) for several species of *Pythium*. In *P. thalassium* growth of evacuation hyphae was rapid. In June 1953 protoplasm was observed passing into the vesicle. At first there was a stream of protoplasm, then, as the rate of flow lessened, distinct units in single file and attached to the following one by a fine thread passed up the hypha into the vesicle. These units were about the size of two zoospores; in the vesicle they at first rounded up individually and could be distinguished from the first flow of protoplasm which formed into a large ball. About 10 min. later the entire contents had separated into the normal-sized zoospores. From the first flow of protoplasm into the vesicle to completion of liberation of the zoospores took about 15 min. Differentiation within the sporangium of *Pythium* has been discussed by Goldie-Smith (1952, pp. 283–6).

No fully formed vesicles were present in preserved material; probably the excessively thin wall of large vesicles bursts on contact with formalin. A few naked balls of protoplasm were found and also a free ball of immature zoospores, as though a vesicle had burst prematurely.

The zoospores are about 15μ long and when encysted 10·5–12·0μ in diameter, measured living.

In the 1929 material extramatrical portions of sporangia frequently had blunt and even swollen ends from which emission tubes of varying length and diameter arose (Fig. 3E–H). When proliferation occurred these globular enlargements were left in the branching system, and in them rounded bodies resembling resting spores, although without thick walls, sometimes remained (Fig. 5). In Fig. 3H two short contiguous sporangia are shown, the distal one with swollen end from which five hyphae had arisen, the proximal one with a single lateral tube.

It was not unusual for small bodies to remain in the sporangium and evacuation tube after the bursting of the vesicle (Fig. 4A). Such bodies either germinated *in situ* or produced miniature tubes of discharge (Figs. 4B, 5); this increased the irregularity of extramatrical growth. In one instance an elongated body remaining in one branch had sent out a hypha which had entered another branch and grown along its length for a certain distance.

Proliferation of the sporangium

Proliferation of the sporangium is known to occur in several members of the genus with spherical sporangia, notably in *Pythium proliferum*, *P. undulatum* and *P. diacarpum*, but seems not to have been described so far in species with filamentous sporangia, whether strictly filamentous or inflated; it is now recorded for *P. thalassium* (Figs. 4, 5). The simplest condition was found when the original tube of discharge projecting from the *Pinnotheres* ovum was short (Fig. 4B, C). The number of times proliferation may occur is unknown; that it may occur apically at least 4 times is evident from the fairly simple example shown in Fig. 4E, and here a lateral emission tube had also been formed. Sometimes the new emission tube did not pass through the old exit, but pierced the wall of the old sporangium in a fresh place (Fig. 4D).

Somewhat complicated growth resulting from proliferation in extramatrical sporangia occurred, and two examples are figured (Fig. 5).

Variation in the behaviour of the contents of the sporangium

A number of variations in the behaviour of the sporangial contents were seen in the 1929 preserved material. This variability may be characteristic of *P. thalassium*, but it is possible, or even probable, that it developed after the egg-carrying pleopods were cut from the crab, for they were allowed to lie on the bottom of the dish and no attempt made to aerate them, although the water was changed frequently: under such conditions bacteria

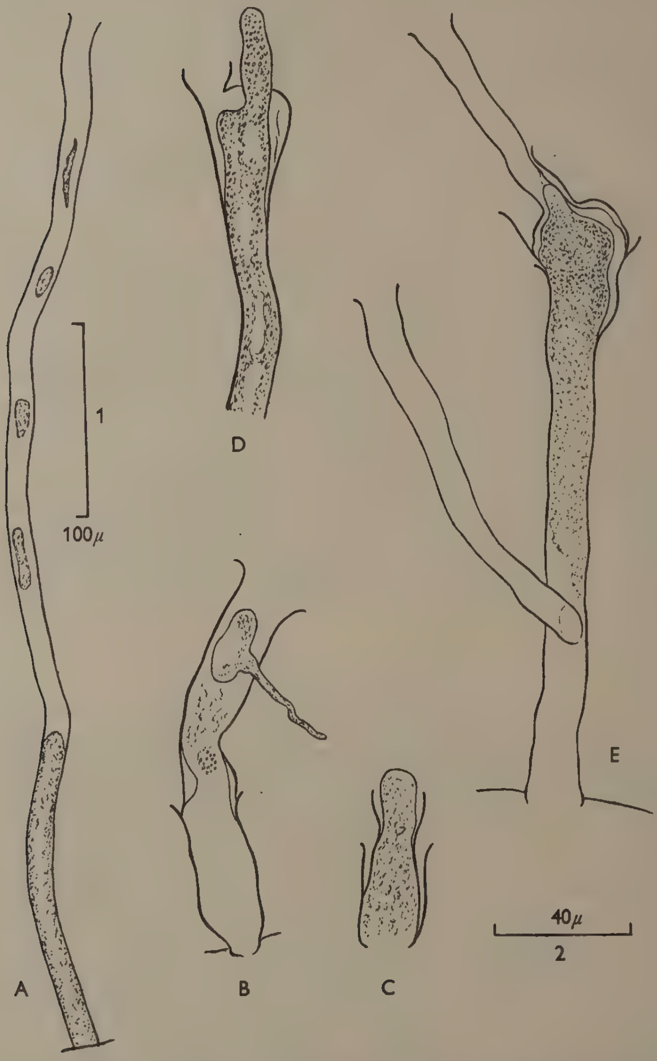

Fig. 4. *Pythium thalassium*. Sporangial proliferation. In A a few small bodies remain in the tube after liberation of the zoospores. B, C, proliferation within short exit tubes; in B, a body in the mouth of the tube has germinated. D, old sporangial wall pierced in a fresh place after renewed growth. E, the apertures of three successive emission tubes are evident, while the hypha shows renewed growth: at some time a lateral exit tube has been formed. A, to scale 1; B–E to scale 2.

and protozoa become troublesome. Normally the crab aerates and cleans the eggs by moving the pleopods back and forth. Certain abnormalities in the behaviour of the sporangium of *P. undulatum* under unfavourable conditions have been described recently by Goldie-Smith (1952, p. 279).

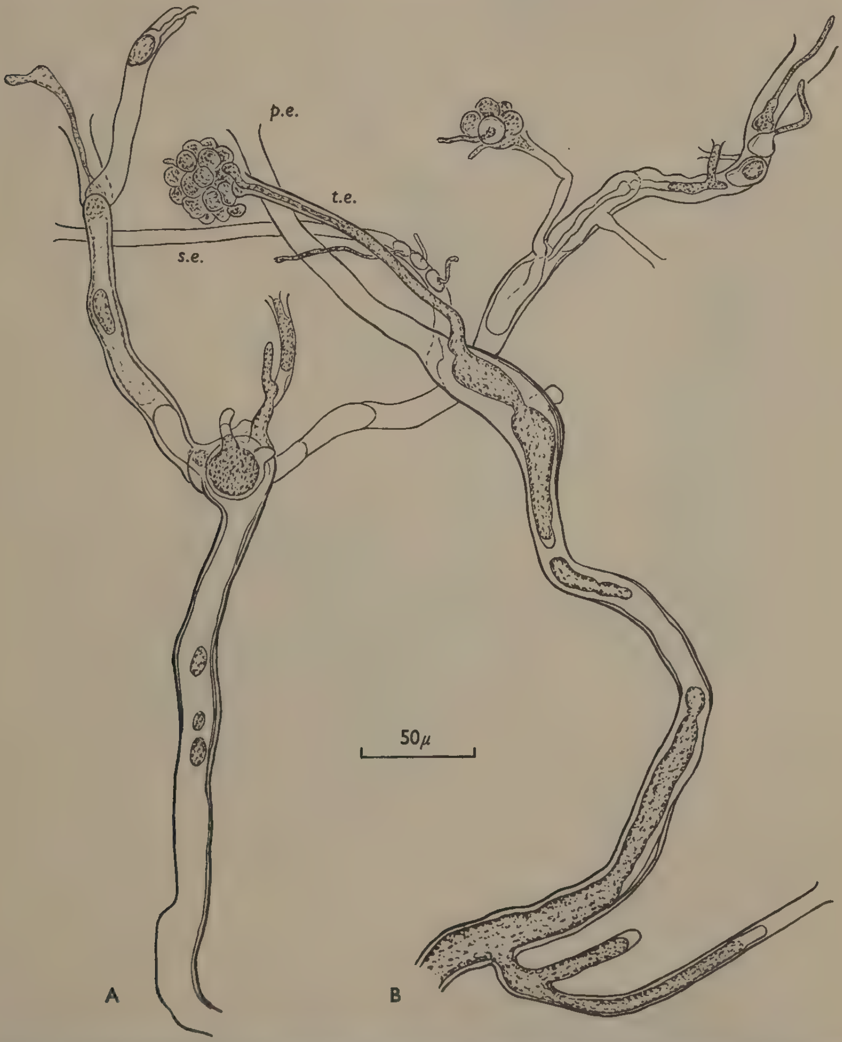

Fig. 5. *Pythium thalassium*. Extramatrical portions of sporangia showing somewhat complicated growth due to proliferation. *p.e.* primary; *s.e.* secondary, and *t.e.* tertiary emission tube.

P. thalassium is a parasite and saprophyte and its normal habitat would never be free of bacteria, although the degree of contamination would vary. If it should prove possible to cultivate it apart from its host in some bacteria-free medium, then under such unnatural conditions its habit of growth may very well be different.

The variations in *P. thalassium* are as follows:

(1) *Encystment and germination of spores within extramatrical portions of a sporangium.* In the example figured (Fig. 6A) the passage of the contents of the sporangium into the vesicle evidently had been incomplete and the dispersal of the zoospores had been inhibited. The spores within the sporangium were unusually large, 15–30 μ in length, and some probably composite. A second very similar example was seen: sometimes only a few spores remain in the sporangium.

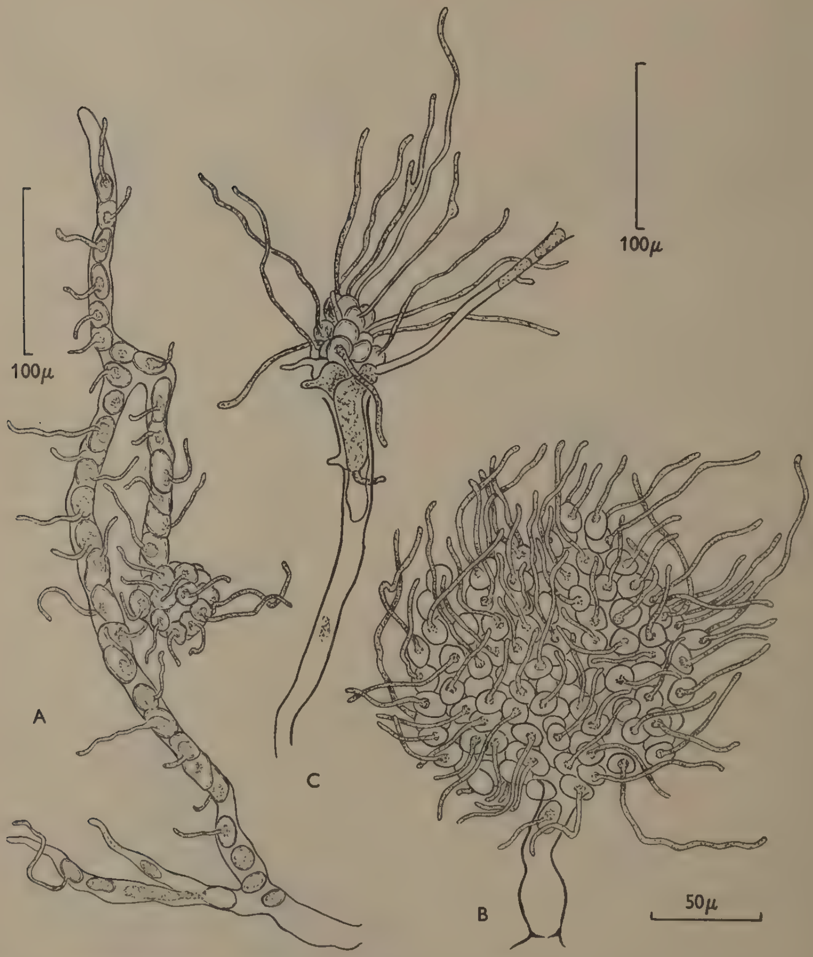

Fig. 6. *Pythium thalassium.* Variation in behaviour of sporangial contents. A: retention and germination of spores in branched extramatrical portion of a sporangium: a small group of germinating spores present at the apex of the emission tube. B: large group of germinating spores at apex of an emission tube. C: cluster of germinating spores of irregular size at apex of an emission tube: a large body in the mouth of the tube has produced an exit tube.

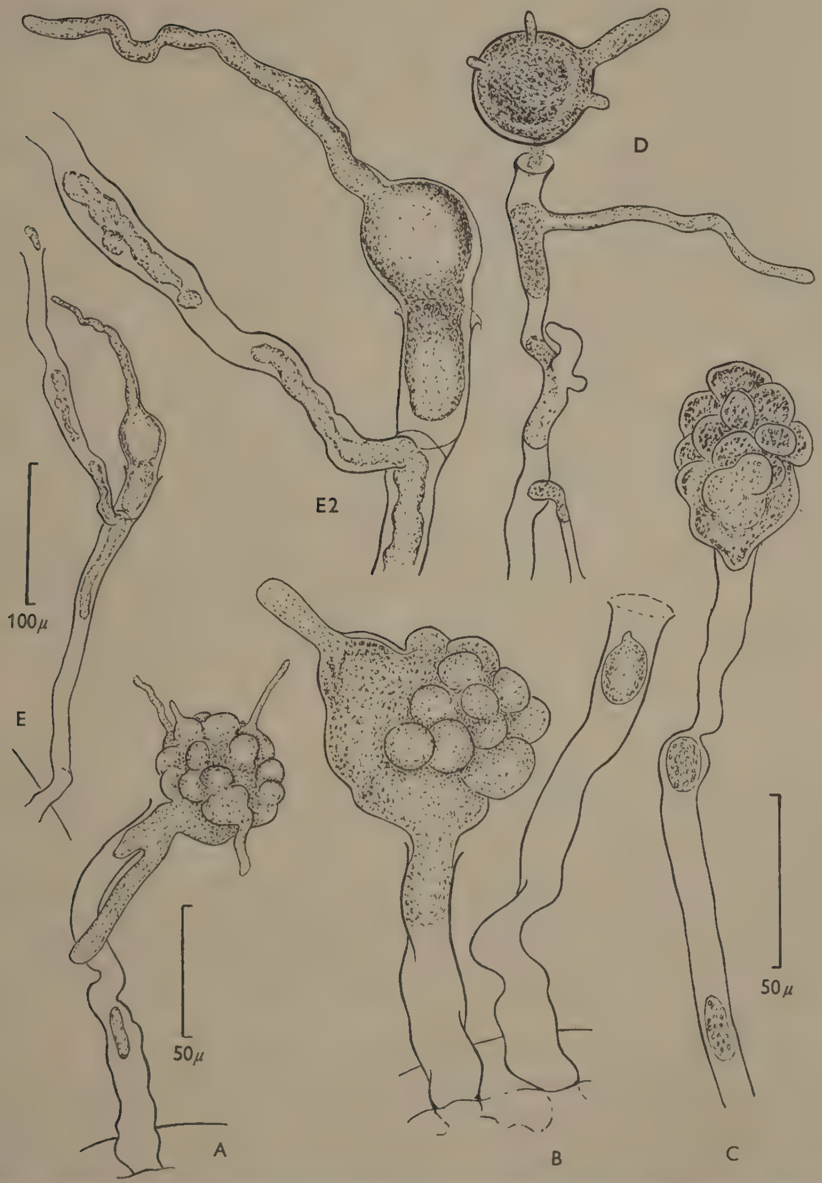

Fig. 7. *Pythium thalassium.* Variation in behaviour of sporangial contents. A, B and C: examples of extruded sporangial masses showing incomplete and irregular division, with, in A and B, production of germ tubes. D: extruded spherical germinating mass attached to apex of sporangium by protoplasmic stalk. E: partially extruded, undivided sporangial mass with germ tube; E2 shows part of E more highly magnified. B–D, E2 to scale on right.

This occasional retention and germination of the spores within the sporangium in *P. thalassium* is of interest in that it is characteristic of the genus *Pythium* that the zoospores are either undifferentiated or imperfectly differentiated within the sporangium, and differentiation is completed in the vesicle. Apparently the only other observation of the formation and germination of zoospores within the sporangium of a true *Pythium* is that of Matthews (1931, pp. 77, 79) who found it to occur in old cultures of *P. pulchrum* v. Minden (a form with spherical sporangia).

(2) *Encystment and germination of spores in a spherical mass at the apex of an emission tube* (Fig. 6B), *following rupture of the vesicle*: the example figured was some 125–130 μ in diameter. In one small cluster (Fig. 6C) the spores were irregular in size and closely packed; an elongated body in the mouth of the tube had itself sent out an emission tube, through which evacuation had occurred. This encystment of spores at the apex of the tube resembles the condition in *Achyla* and especially in *Aphanomyces* (Saprolegniaceae), but in these two genera the cysts give rise to zoospores.

(3) *Extrusion of sporangial contents, not followed by division into spores.* (*a*) Extrusion and germination of incompletely divided sporangial contents. In two instances figured (Fig. 7A, B) the mass had apparently been contained in a vesicle, which ruptured prematurely, as indicated by the flared mouth of the emission tube below the mass, but sometimes the formation of a vesicle was not evident (Fig. 7C). (*b*) Extrusion and germination of undivided sporangial contents (Fig. 7D, E). In one example (Fig. 7D) the mass, 32 μ in diameter, had become rounded off, the only attachment to the sporangium being a slender stalk of protoplasm.

Asexual bodies

Spherical to pyriform asexual bodies, 25–52 μ in diameter, occasionally occurred on unbranched hyphae resembling evacuation tubes (cf. Vanterpool, 1938, p. 533, on *Pythium tardicrescens*), but these bodies were distinguished from vesicles by the greater thickness of the containing wall (Fig. 8). The hyphae below such bodies were sometimes entirely empty, sometimes contained protoplasm of a variable length.

Few of these bodies have been seen germinating; that figured (Fig. 8A) was borne on a hypha which showed spiral coiling; it was the only extramatrical growth of a small mycelium in an egg shell from which the zoea had hatched.

In the group with filamentous sporangia similar bodies in *P. afertile* have been termed chlamydospores by Kanouse & Humphrey (1928), while Matthews (1931) considered them to be conidia, as also in *P. perniciosum* (with strictly filamentous sporangia), *P. catenulatum* and *P. graminicolum* (with inflated sporangia). Vanterpool (1938) has recorded conidia in *P. tardicrescens* and *P. aristosporum*, two species with inflated sporangia. Middleton (1943), on the other hand, held that true conidia do not occur in the genus *Pythium*: he suggested that in some instances they are probably frustrated oogonia (see also Tompkins, Ark, Tucker & Middleton, 1939, p. 467) and in others sporangia germinating by means of germ tubes.

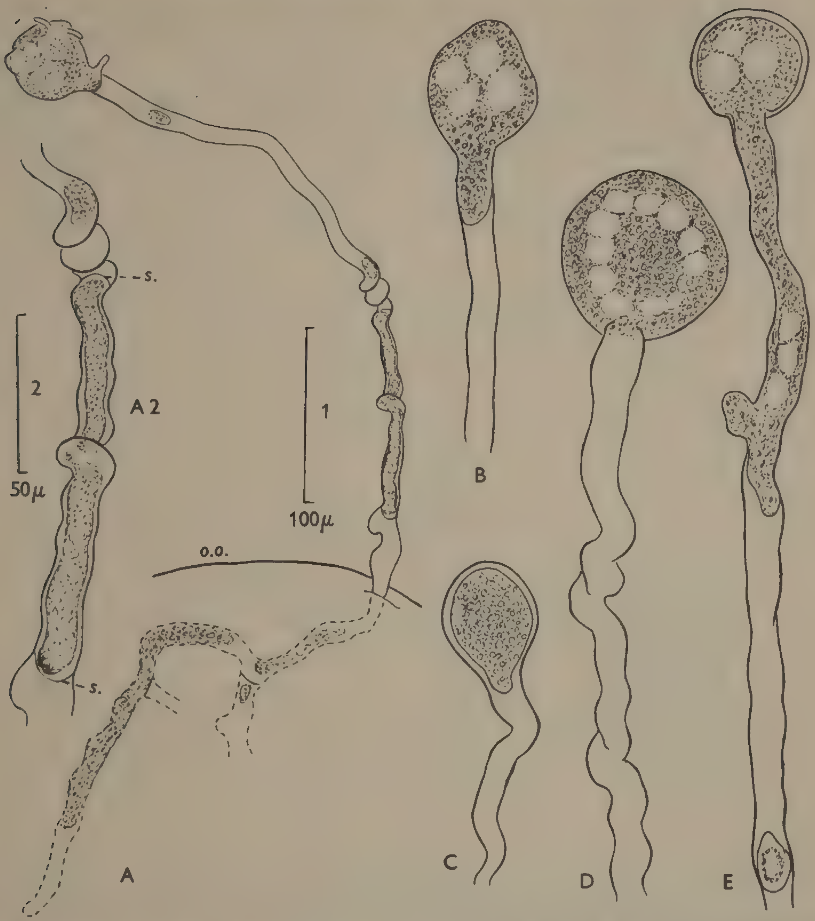

Fig. 8. *Pythium thalassium.* Asexual bodies on hyphae of varying length: large vacuoles are present in most of them. A was the only extramatrical growth from a small mycelium in an egg envelope, from which the zoea had hatched: A2 part of hypha of A exhibiting spiral formation, more highly magnified. *o.o.* outline of *Pinnotheres* ovum; *s.* septum; intramatrical mycelia indicated by broken line. A to scale 1; A2, B–E to scale 2.

Germination of spores and entry into the Pinnotheres *ovum*

Numerous *Pinnotheres* ova have been observed with germinating spores lying on their surface. The germ tubes from many had effected entrance, sixty being counted on the half of one ovum, but curiously enough the tubes from many spores grew away from the egg: this may possibly be due to the close proximity of other eggs in the egg mass. Germ tubes growing freely in sea water remained fine, as they lacked nutriment.

Owing to the opacity of the yolky *Pinnotheres* ovum the entry of the fungus could be clearly seen only in ova in which little yolk remained. In one such ovum the fine tube put out by spores lying on the surface had pierced the egg membranes and inside had expanded into a hypha, into which the spore contents had passed leaving the empty cyst outside (Fig. 9). The contents of the young hyphae showed shrinkage, possibly caused by the preservative.

A very young mycelium is shown in Fig. 9C: it was growing just beneath the egg envelopes of an ovum almost filled with yolk. The cyst, coated with bacteria and empty except for two tiny granules, is still attached.

Cysts in preserved material varied in thickness of wall.

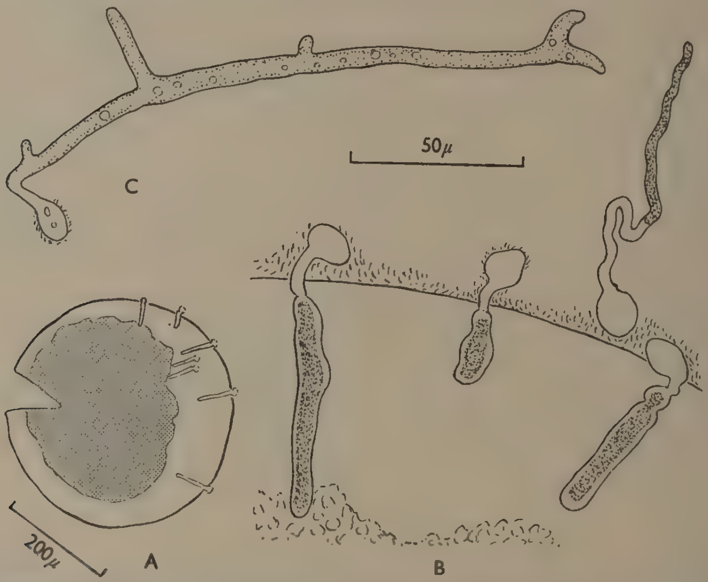

Fig. 9. *Pythium thalassium.* A: invasion of *Pinnotheres* egg (burst by pressure on cover-slip) by a number of germinating spores. The egg was already degenerate, with yolk mass reduced. B: invading spores, and one growing away from the egg surface, shown enlarged; bacteria coated cysts and surface of ovum. C: young mycelium from beneath surface of an egg; the empty cyst is coated with bacteria.

Sexual reproduction

Sexual reproduction has not yet been seen. In 1953 *Pythium thalassium,* kept at a constant temperature of 15–16° C. for over 3 months, failed to produce oogonia. Possibly the asexual bodies previously described (p. 42) are frustrated oogonia, as suggested by Middleton (1943, pp. 27, 44) for similar structures found in certain other species. But asexual bodies are formed extramatrically in *P. thalassium*, while sexual organs are more frequently formed intra- than extramatrically in aquatic species of *Pythium.*

AFFINITIES

P. thalassium differs from all known species of the genus with filamentous sporangia (including those with strictly filamentous and those with inflated sporangia) in exhibiting renewal of the sporangium by proliferation, and from all except *P. marinum* Sparrow in its marine habitat.

It differs from *P. marinum* Sparrow in: (1) coarseness of habit and richness of branching; (2) considerably greater number and larger size of zoospores, about 15 μ long as against 7·5 μ in *P. marinum*; (3) growing on an animal and not a vegetable substratum.

Among species with strictly filamentous sporangia it agrees with *P. papillatum* Matthews in coarseness of hyphae, and with *P. afertile* (Kanouse & Humphrey, 1928) and *P. perniciosum* (Matthews, 1931) in the possession of extramatrical rounded asexual reproductive bodies.

Sexual reproduction is unknown at present, but as no culturing has been done it cannot be stated that *P. thalassium* lacks a sexual stage as was claimed for *P. afertile* by Kanouse & Humphrey (1928).

P. thalassium would appear to be a true *Pythium* and not a member of the Lagenidiales such as described by Couch (1942), Newcombe & Rogers (1947) and Rogers-Talbert (1948) from the eggs (0·25 mm. in diameter) of the blue crab (*Callinectes sapidus*) from off Lynnhaven, Virginia under the name of *Lagenidium callinectes*. There are a number of points of difference: the vesicle in *Pythium thalassium* is evanescent and the zoospores swim actively away, as is typical of *Pythium*; while in *Lagenidium callinectes* the zoospores swim sluggishly away and the vesicle persists for several hours after their escape (Couch, 1942). Proliferation of the sporangium has not been described in *L. callinectes*. The mycelium in *Pythium thalassium* is more richly branched than in *Lagenidium callinectes*. Extramatrical asexual bodies are present in *Pythium thalassium*, while in *Lagenidium callinectes* resting bodies are intramatrical. Couch has described in *L. callinectes* short exit tubes, up to 70 μ long, which much resemble the short exit tubes found on some *Pinnotheres* ova infected with *Pythium thalassium*. Newcombe & Rogers (1947) state that, regarding the appearance of the fungus on and in the individual egg, it may be confined almost entirely to the interior or show up noticeably on the outside, depending on the stage of development.

I am indebted to Dr W. R. G. Atkins, C.B.E., F.R.S., for the interest he has taken in the work and to Dr B. Barnes for reading the manuscript. Dr J. Morton has most kindly provided the Latin diagnosis.

REFERENCES

Atkins, D. (1929). On a fungus allied to the Saprolegniaceae found in the pea-crab *Pinnotheres*. *J. Mar. biol. Ass. U.K.*, N.S., **16**, 203–219.

Atkins, D. (1954). Further notes on a marine member of the Saprolegniaceae, *Leptolegnia marina* n.sp., infecting certain invertebrates. *J. Mar. biol. Ass. U.K.*, N.S., **33**, 613–625.

Brown, W. (1948). A discussion on the physiology of resistance to disease in plants: physiology of the facultative type of parasite. *Proc. Roy. Soc.* B, **135**, 171–179.

Butler, E. J. (1907). An account of the genus *Pythium* and some Chytridiaceae. *Mem. Dep. Agric. India, Bot.*, **1**, 1–160.

Couch, J. N. (1942). A new fungus on crab eggs. *J. Elisha Mitchell sci. Soc.* **58**, 158–162.

Goldie-Smith, W. K. (1952). The sporangial phase of *Pythium undulatum* Petersen. *J. Elisha Mitchell sci. Soc.* **68**, 273–292.

Kanouse, B. B. & Humphrey, T. (1928). A new species of the genus *Pythium* in the sub-genus *Aphragmium*. *Pap. Mich. Acad. Sci.* **8**, 129–140.

Matthews, V. D. (1931). *Studies on the genus* Pythium. University of North Carolina Press. 136 pp.

Middleton, J. T. (1943). The taxonomy, host range and geographic distribution of the genus *Pythium*. *Mem. Torrey bot. Cl.* **20**, 1–171.

Newcombe, C. L. & Rogers, M. R. (1947). Studies of a fungus parasite that infects blue crab eggs. *Turtox News*, **25**, no. 9.

Petersen, H. E. (1910). An account of Danish Freshwater Phycomycetes, with biological and systematic remarks. *Ann. mycol., Berl.*, **8**, 494–560.

Rogers-Talbert, M. R. (1948). The fungus *Lagenidium callinectes* Couch (1942) on the eggs of the blue crab in Chesapeake Bay. *Biol. Bull., Woods Hole*, **95**, 214–228.

Sandoz, M. D., Rogers, M. R. & Newcombe, C. L. (1944). Fungus infection of eggs of the blue crab *Callinectes sapidus* Rathbun. *Science*, **99**, 124–125.

Sparrow, F. K. (1931). Two new species of *Pythium* parasitic in Green Algae. *Ann. Bot., Lond.*, **45**, 257–277.

Sparrow, F. K. (1934). Observations on marine Phycomycetes collected in Denmark. *Dansk. Bot. Arkiv.* **8**, 1–24.

Tompkins, C. M., Ark, P. A., Tucker, C. M. & Middleton, J. T. (1939). Soft rot of pumpkin and watermelon fruits caused by *Pythium ultimum*. *J. agric. Res.* **58**, 461–475.

Vanterpool, T. C. (1938). Some species of *Pythium* parasitic on wheat in Canada and England. *Ann. appl. Biol.* **25**, 528–543.

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Trans. Brit. mycol. Soc. **38** (1), 47–48 (1955).

HARPOSPORIUM CRASSUM SP.NOV.

By AUDREY M. SHEPHERD
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(With 1 Text-figure)

A species of *Harposporium*, *H. crassum*, with a rather coarse mycelium and crescent-shaped conidia, is described for the first time. An internal parasite of nematodes, the fungus was found in horse dung from Denmark.

Harposporium crassum sp.nov. (Fig. 1)

Hyphis in corpore vermiculi (Nematoda) ramosis hyalinis, 5·5–6·5 μ crassis, ad septa conferta leniter constrictis. Hyphis e corpore protrudentibus fertilibus subcylindricis, 50–150 × 1·5–2·5 μ, ramos conidiophoros usque ad 7, subglobosos 2·5–4·5 μ crassos gerentibus. Conidiis 18–22 μ × 2–3 μ, hyalinis curvatis aseptatis e ramo subglobosa unicis.

Vermiculos consumens, habitat in fimo equi, Ågård, Dania, Februario 1953.

Six weeks after inoculating a maize-meal agar plate with a sample of horse dung used for mushroom-growing from Ågård, Denmark, this fungus appeared killing some of the eelworms which had emerged from the inoculum. The fungus consisted of an endozoic mycelium of branched, septate hyphae, 5·5–6·5 μ wide, the cells of which were rather short and broad and somewhat thick-walled (Fig. 1 *c*). From the endozoic mycelium, and at right angles to it, were produced fine septate branches which emerged through the cuticle of the dead eelworm and extended for a distance of from 50 to 150 μ into the surrounding medium. These septate branches were 1·5–2·5 μ in width and they gave rise to small, unicellular, subspherical fertile branches, 2·5–4·5 μ in diameter, on which the conidia were borne (Fig. 1 *a*).

The conidia, arcuate in form, were 18–22 μ long and 2–3 μ wide and were produced on small protuberances from the tiny fertile branches (Fig. 1 *a*, *b*).

No living eelworms were observed with conidia attached to them and degenerating tissues within the eelworm obscured the early stages of mycelial development. However, the form of the fertile branches and the conidia are characteristic of the genus *Harposporium* Löhde and there is no doubt that this fungus can be placed in that genus.

The conidia resemble to a great extent those of *H. anguillulae* Löhde (1874), but are significantly larger. In length they are within the range described by Drechsler (1941) for *H. oxycoracum*, but they are much wider and lack the basal swelling which is a character of the conidia of *H. oxycoracum*. The endozoic mycelium is also considerably coarser than in any of the species of *Harposporium* so far described. The aerial branches and the globose fertile branches show little variation in size amongst the various species within this genus.

Because of the large size of the conidia and the width of the endozoic mycelium, it is proposed to erect a new species, *Harposporium crassum*, the epithet meaning coarse.

The type material is deposited in the herbarium of the Commonwealth Mycological Institute, Kew. Measurements were made from living material and drawings from preparations stained with cotton blue in lactophenol.

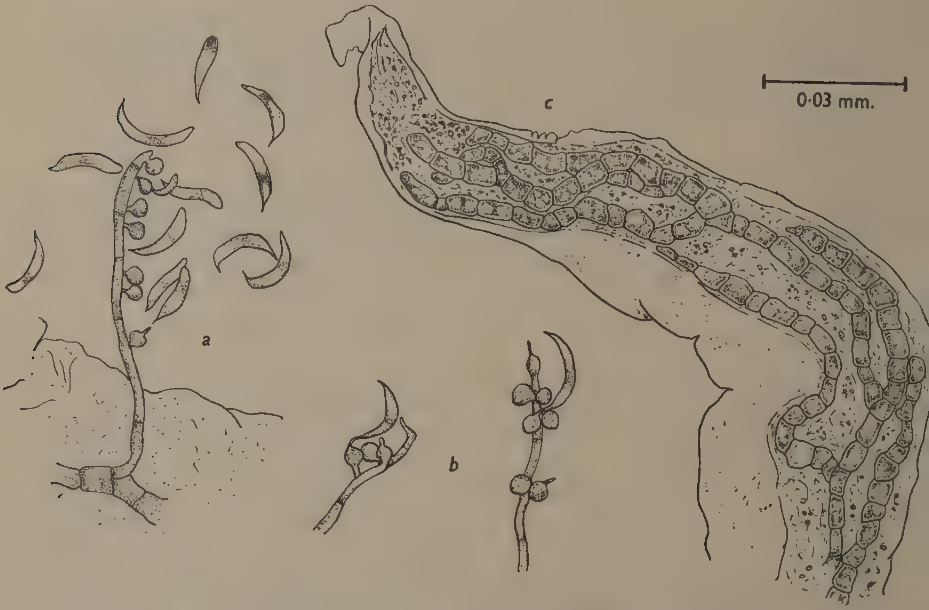

Fig. 1. *Harposporium crassum* sp.nov. *a*, *b*, fertile hyphae bearing conidia; *c*, mycelium within the eelworm.

I should like to express my thanks to Mr Knud Lindhardt for providing the material and to Dr C. L. Duddington, Dr E. J. H. Corner and Mr Henrik A. Jørgensen for their assistance in the preparation of this paper. The work was carried out in Copenhagen, at Statens plantepatologiske Forsøg, Lyngby, under a British Council Scholarship.

REFERENCES

Drechsler, C. (1940). Three fungi destructive to free-living terricolous nematodes. *J. Wash. Acad. Sci.* **30**, 240–254.

Drechsler, C. (1941). Some hyphomycetes parasitic on free-living terricolous nematodes. *Phytopathology*, **31**, 773–802.

Löhde, G. (1874). Einige neue parasitische Pilze. *Tageblatt der* 47. *Versammlung deutscher Naturforscher und Ärtze in Breslau*, pp. 203–206.

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THE CYTOLOGY AND RELATIONSHIPS OF *CORYNEBACTERIUM FASCIANS*

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(With Plate 1 and 4 Text-figures)

Corynebacterium fascians, grown on a synthetic medium of low nitrogen content, showed marked cytological differences from cultures on peptone media. The cells varied from coccoid forms to long, unsegmented filaments very similar to the growth of *Nocardia*. Reproduction by the cutting off of 1 to 6 or more cocci in a chain from one end of a long rod was frequent. The cocci and the tip of the parent cell from which they were derived stained deeply with methyl violet; the rest of the parent cell was either very faintly stained or unstained except for scattered granules.

Acid-fast bodies, sometimes completely filling the cells, developed in twenty-nine of thirty pathogenic strains of *Corynebacterium fascians* grown on the synthetic medium. Of four pink, non-pathogenic cultures, two were feebly acid-fast and two were non-acid-fast. *C. fascians* cultures grown on a variety of peptone media were invariably completely non-acid-fast.

INTRODUCTION

Corynebacterium fascians (Tilford) Dowson, the causal agent of fasciations and leafy galls on a variety of host plants, is a yellow, non-motile, Gram-positive rod and is invariably non-acid-fast on peptone media.

A study was made of the growth of *C. fascians* on a variety of culture media and it was found that when the organism was grown on a synthetic medium of low nitrogen content the cells exhibited marked differences from the normal morphology. The study of growth on this medium was therefore extended, thirty pathogenic and four non-pathogenic strains of *C. fascians* in all being examined.

The synthetic medium had the composition: sucrose or mannitol, 10 g.; K_2HPO_4, 0·5 g.; $MgSO_4 . 7H_2O$, 0·2 g.; NaCl, 0·2 g.; KNO_3, 0·5 g.; $CaCO_3$, 3 g.; 'Yeastrel', 1·0 g.; agar, 15 g. in 1 l. of tap water. Cultures grown on this medium were compared with those grown on nutrient agar of the composition 'Lab-Lemco', 5 g.; peptone, 10 g.; NaCl, 5 g.; agar, 15 g. in 1 l. tap water. All cultures were incubated at 25° C.

The cells were usually examined by mounting the living cells in dilute methyl violet and observing the gradual penetration of the dye. Methylene blue, Kopeloff & Beerman's modification of Gram's method, and Shaeffer & Fulton's malachite green and safranin stain for spores were also used.

The Ziehl-Neelsen technique was used for the acid-fast stain. Lipid material was demonstrated by staining with Sudan Black with methylene blue counterstain.

C. fascians cultures. The thirty-four strains included in this work were isolated at various times from 1935 to 1950 from abnormal growths on the following plants: sweet-pea (3 strains); chrysanthemum (3); strawberry (8); verbascum (4); wall-flower (3); dahlia (3); *Nicotiana tabacum* (2); primula (2); heuchera, sugar beet, phlox, potato, petunia and polyanthus (1 each). Thirty of the strains produced typical galls when inoculated on to sweet-pea seedlings shortly after isolation; some lost their power to induce gall-formation after long periods of growth on artificial media. Four strains, otherwise identical in morphological and physiological characters with the thirty pathogenic strains, were pink instead of yellow and were avirulent on first isolation. They occurred on the same isolation plates as the yellow, virulent colonies.

The preliminary, and most extensive, observations were made on strain 505 (isolated from sweet-pea, 1948). On nutrient peptone agar this strain invariably produced a mixture of rough and smooth colonies. Six other strains were also examined in detail: str. 12, rough (sweet-pea, 1935); str. 444, rough (chrysanthemum, 1938); str. 2 W, rough (a white variant from wall-flower, 1946); str. 520, smooth; and str. 521, smooth (both isolated from strawberry plants with 'cauliflower' disease by Dr Crosse, East Malling, 1950). The remaining twenty-seven strains were studied principally for their reaction to acid-fast staining. All the strains were closely similar in cultural characteristics in spite of the wide variety of the host-plants and the considerable differences in the time they had been kept on artificial media.

OBSERVATIONS

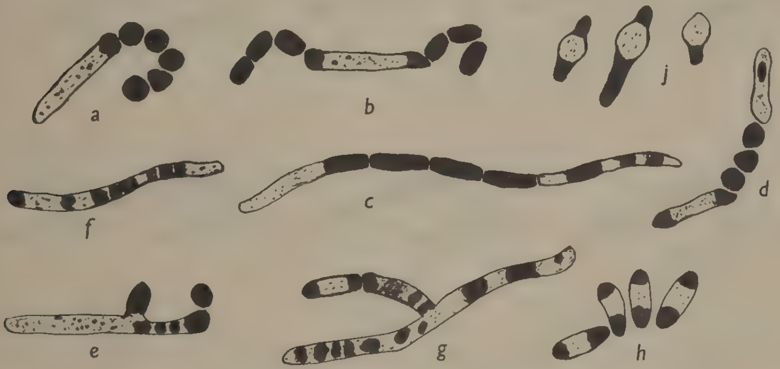
Macroscopical examination

Cultures of *C. fascians* on peptone media showed a sharp differentiation between 'rough' and 'smooth' types of growth. 'Rough' colonies were large, flat, dull, dry and very wrinkled, whereas 'smooth' colonies were much smaller, with entire margins, raised, smooth, butyrous and shining. When grown on the special synthetic medium, however, all the strains produced a copious, mucoid, glistening, light yellow growth with no differentiation into 'rough' and 'smooth' types. Colonies on this medium were invariably smooth even when subcultured from strains, such as str. 12, that had produced all rough colonies on peptone agar for several years. When these colonies were transferred back to peptone medium the rough character reappeared.

Microscopical examination

Nutrient peptone agar cultures 2–15 days old consisted of rods of fairly uniform length growing in characteristic X, W or Y formations. No branching filaments or coccoid forms were seen and multiplication was by snap fission.

On the nitrogen-deficient medium the majority of the cells in a 2-day-old culture were short rods which stained deeply at one pole when mounted in dilute methyl violet; there were also a few coccoid forms. Three-day-old cultures contained large numbers of deeply stained cocci which were produced from the tip (also deeply stained) of a long rod or filament. These cocci frequently formed chains of two to six or more still attached to the parent rod (Text-fig. 1 *a*). Occasionally, cocci or ellipsoidal cells were produced from both poles of a rod, or a bud was produced laterally. In others the centre of a filament fragmented into short rods or cocci (Text-fig. 1 *b–e*). When films from a 5-day-old culture were stained by Gram's method the parent rods were pink, except for scattered purple granules and one deep purple pole to which a chain of strongly Gram-positive cocci was attached. Long rods or filaments sometimes showed deeply stained purple bands or irregular masses alternating with pink or unstained areas (Text-fig. 1 *f, g*). In older (6- to 12-day-old) cultures the predominant forms were short rods which developed from the coccoid forms. These rods exhibited bi-polar staining, the centre of the cell being unstained (Text-fig. 1 *h*).

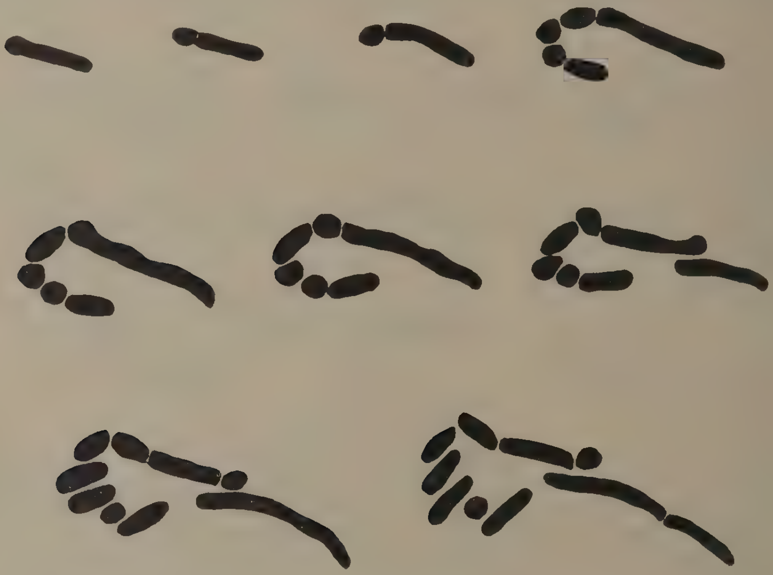


Text-fig. 1. Cells of 3- to 5-day-old cultures of *C. fascians* stained with dilute methyl violet or Gram's stain. *a*, chain of cocci from one pole of rod; *b*, ovoid cells produced from both ends of a long rod; *c* and *d*, filaments fragmenting into short rods or cocci; *e*, lateral bud production; *f* and *g*, Gram-positive banding of long rods or filaments; *h*, bi-polar staining of short rods; *j*, cells showing unstained swollen areas.

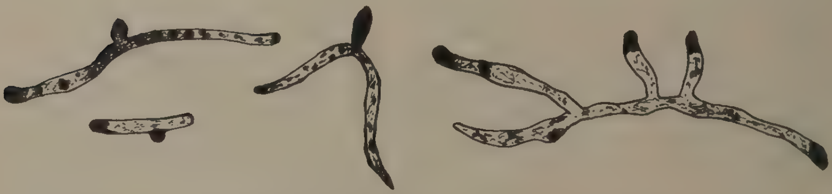
The growth of a single cell and the production of cocci was observed over a period of 30 hr. by placing cells from a 4-day-old culture on a thin film of synthetic medium on a cover-slip mounted on a cavity slide. A field containing a few rods of medium length was selected under the microscope, and drawings of their growth and division made at intervals. The diagrams (Text-fig. 2) show the production of a chain of cocci, with some elongated into short rods, from one pole of a rod. After 27 hr. the parent rod underwent normal division into two, and this was followed by the cutting off of a coccus from each end of the active half. No motility was ever observed either of cocci or rods. Two of the strawberry strains (520 and 521) were especially pleomorphic on the synthetic medium, the cells varying from cocci to long branching filaments which closely resembled the growth of an Actinomycete (Text-fig. 3; Pl. 1, fig. 1). No transverse walls were ever seen in these filaments, or in shorter rods in any of the strains. The central part or one end of a rod or filament was frequently swollen and bulbous, the appearance being that of a large spore (Text-fig. 1 *j*), but tests for spores gave negative results.

Acid-fast staining

In the preliminary examinations of str. 505 grown on the synthetic medium a number of round, spore-like bodies were frequently seen in the cells. These bodies stained deeply with spore stains but the cultures in which they occurred showed no increase in heat resistance. Further tests showed that the bodies were strongly acid-fast when stained by the Ziehl-Neelsen technique.

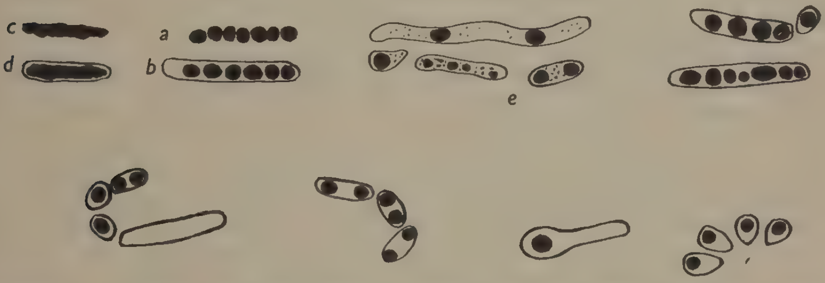


Text-fig. 2. *C. fascians*. Coccoid cells from a rod during 30 hr. growth.



Text-fig. 3. *C. fascians* (str. 520; strawberry). Actinomycete-like growth.

A comparison was then made of str. 505 and six other strains grown on the synthetic medium and on nutrient peptone agar, films of the cultures from each medium being made on the same slide and stained for acid-fastness. Invariably, in all the strains and at all ages up to 6 weeks or more, cultures on nutrient agar were completely non-acid-fast, this being in agreement with the numerous tests for acid-fastness that had previously been made on *C. fascians* cultures. On the contrary, all the seven cultures on the synthetic medium developed conspicuous acid-fast bodies, usually first apparent about the 6th day of growth. In the acid-fast staining the methylene-blue counter-stain was scarcely, or not at all, taken up by the cell once the acid-fast bodies had been formed. As these lay in a row throughout the length of the cell the appearance was that of a short chain of red streptococci (Text-fig. 4*a*; Pl. 1, fig. 3). When a nigrosine background was added after the completion of the staining a clear picture was obtained of unstained cells, varying from cocci to long rods or short filaments, each containing one to six or more acid-fast bodies (Text-fig. 4*b*). (For comparison, Text-fig. 4*c* and *d* show acid-fast staining of *Mycobacterium phlei* cells, (*c*) without and (*d*) with, a nigrosine background.) Coccoid or ellipsoidal forms were either completely acid-fast or, more commonly, contained one acid-fast bead, short rods usually had an acid-fast bead at each pole and long rods or filaments were frequently filled with a row of acid-fast beads (Text-fig. 4; Pl. 1, figs. 2, 4 and 5). The acid-fast character of these beads was compared with that of *M. phlei* and found to be of equivalent strength. Films of the two organisms were made on the same slide and, after staining with hot carbol-fuchsin, were washed for varying lengths of time with acid alcohol or with different concentrations of various acids. In every case the *C. fascians* cells retained the red stain fully as long as the *M. phlei* cells.



Text-fig. 4. *C. fascians*. Acid-fast bodies: *a*, chain of acid-fast beads, no cell-wall visible; *b*, acid-fast beads in a rod, shown up by a nigrosine background; *c* and *d*, acid-fast staining of *Mycobacterium phlei* (*c* without, and *d* with, a nigrosine background); *e*, small acid-fast granules in addition to large beads, seen in some cells.

Tests for acid-fastness were then made on twenty-three more pathogenic *C. fascians* strains, and four pink, non-pathogenic strains grown on the synthetic medium; twenty-two of the former and two of the latter developed acid-fast bodies. Thus of the thirty-four strains examined for acid-fastness, thirty-one were positive.

The age of the culture in which the acid-fast bodies first appeared, their frequency, size and the number in each cell, varied with the different strains. Cultures 1–3 days old were invariably negative. On the 4th day a few acid-fast beads were observed in three strains and these and thirteen others were strongly positive on the 5th to 9th days. In six strains acid-fast material was first seen on the 12th day and in the remaining nine strains the production was delayed until the 15th day. The three negative strains were examined at intervals up to 6 weeks but were invariably completely non-acid-fast.

Twenty-seven of the positive strains developed large acid-fast bodies in the great majority of the cells but the other four were only feebly acid-fast, a small quantity of acid-fast material being present in some of the cells of 15-day-old cultures; two of those were pink non-pathogenic strains. The one typical *C. fascians* strain which was negative was str. 12, rough (sweet pea, 1935); str. 12, smooth, was one of the very feebly acid-fast strains. Both these strains, which were strongly virulent for years after isolation, had latterly become non-pathogenic.

In some cultures the acid-fast material was of two types: (1) large, round beads with a clearly defined margin which were strongly acid-fast; and (2) small granules of indefinite size and shape, in groups or scattered throughout the cell. These were less strongly acid-fast than the beads. Sometimes both types were present in the same cell (Text-fig. 4*e*; Pl. 1, fig. 5).

Yegian & Baisden (1942) found that when films of *Mycobacterium tuberculosis* were stained by the Ziehl-Neelsen technique, the addition of 0·5 % sodium chloride to the carbol-fuchsin caused intense beading of the cell contents, 1 to 5 dark purple beads (looking like a chain of cocci) being present in each cell, the body of which was stained very faintly pink. If, however, after decolorizing in acid-alcohol, the slides were washed in 95 % ethyl alcohol alone, the beading disappeared and the bacilli showed even pink staining throughout. Tiny granules which were seen in tubercle bacilli stained by ordinary carbol-fuchsin did not disappear when washed with neutral alcohol and appeared therefore to be of a different nature from the beads. The deeply stained beads and lighter stained small granules of *M. tuberculosis* described by Yegian & Baisden appear very similar to the two types of acid-fast material constantly seen in *C. fascians* cultures grown on the synthetic medium, except that the former are observed in films stained with carbol-fuchsin without the addition of salt. Cultures of *M. phlei* and two strains of *C. fascians* (503 and 520) were grown on the synthetic medium, and stained by Yegian & Baisden's method. The *M. phlei* cells were stained a brighter, clearer red colour than usual but did not exhibit marked beading and washing with neutral alcohol had no effect. In the *C. fascians* cells the large beads were stained very deeply; in str. 503 the two types of acid-fast material were clearly differentiated, the deep purple beads contrasting with small pink granules of indefinite size and shape. When *C. fascians* cells were further washed with neutral alcohol some of the deep red dye diffused from the beads into the body of the cell which was stained red throughout. The beads remained visible, however, and showed as deep red bodies in a lighter red rod, giving a beaded appearance very like that often seen in acid-fast stained films of *M. tuberculosis*.

The staining of cells of *M. phlei* and *C. fascians* (str. 520) by Sudan Black with methylene blue counterstain was examined. In *M. phlei* films the body of the cell was stained pale blue and contained numerous small black granules. The *C. fascians* cells were also stained pale blue and the large, acid-fast beads were stained deep black. This affords evidence of the lipid nature of these bodies.

In addition to *C. fascians* and *M. phlei*, cultures of *Rhizobium* sp., *Bacillus polymyxa*, *Xanthomonas campestris* and *C. michiganense* were grown on the synthetic medium and examined for acid-fast bodies. No acid-fast material was formed by the first three organisms at any stage in their growth, but *C. michiganense* resembled *C. fascians* in producing acid-fast bodies, first apparent on the 8th day of growth. Unlike *C. fascians*, *C. michiganense* was not pleomorphic on the synthetic medium, the cells being mostly rather short rods, the great majority of which contained one, or sometimes two, acid-fast bodies situated at one or both poles.

Discussion

The inclusion of *C. fascians*, *C. michiganense* and other Gram-positive plant pathogens in the genus *Corynebacterium* by Dowson (1942) has met with some criticism (Starr & Weiss, 1943; Conn, 1947; Conn & Dimmick, 1947). Mohanty (1951) investigated the morphology, physiology and staining properties of twenty-two strains of *C. fascians* and concluded that the placing of this organism in the genus *Corynebacterium* is largely because of the wide range of certain characteristics of that genus. Mohanty says: 'Bisset & Moore (1949) have studied the "soil diphtheroids" which branch only occasionally and have their branches separated from the parent cell, and have shown that they are really unicellular. On that ground these authors would separate them from the multicellular types. The genus *Jensenia* has been proposed to accommodate them, and in this the fasciation organism, if found to be unicellular, might well be placed. However, *Jensenia* is proposed as a genus of the order Actinomycetales....' Mohanty considered that further evidence was necessary before the fasciation organism was included in the Actinomycetales.

Although the macroscopical appearance of colonies and the type of growth of some *C. fascians* strains often resembled those of the genus *Nocardia*, until the observations recorded in the present paper no branching filaments, budding or cocci had been seen. Some species of *Nocardia* are acid-fast but Jensen (1953) says: 'Many species can probably best be described as potentially acid-fast, because the acid-fastness may be apparent for only a very brief period in the organism's life history and may be induced by special media, like milk or synthetic media with paraffin or high concentrations of glycerol.' In this character *C. fascians* closely resembles *Nocardia* spp., for the acid-fastness is only developed when the fasciation organism is grown on a special medium. Also, on this medium, *C. fascians* becomes very pleomorphic, producing short, sometimes branched, filaments, rods of various lengths and coccoid forms. These cells are remarkably similar to those of *Nocardia* spp. as described by McClung (1950), in fact his photographs of *Nocardia* might well represent *C. fascians* cells grown on the special medium. The relationship between *C. fascians* and *Nocardia* is emphasized by the report of Demaree & Smith (1952) that a *Nocardia* (*N. vaccinii*) is the causal agent of a bud-proliferating gall on blueberry. The description and illustrations of this gall, which arises on the stem at or immediately below ground-level, and produces numerous abnormal buds which may abort or grow into short, thin, spindly shoots, are remarkably like the galls produced by *C. fascians* on, for example, chrysanthemum or sweet-pea. The chief difference is that an injured surface is necessary for infection by *N. vaccinii* to take place, whereas *C. fascians* causes gall production when present on the surface of uninjured, healthy bud tissue.

In the genus *Corynebacterium*, *C. fascians* has many characters in common with *C. equi*, a species in which Brooks & Hucker (1944) include all strains of diphtheroids which are actively pathogenic to animals, particularly horses and cattle. *C. equi* is exceptional among the Corynebacteria in that it shows acid-fast staining of cells especially when grown on special media, such as milk. The cells vary from long curved rods to coccoid or ellipsoidal forms according to the medium on which they are grown. Most of the coccoid forms are completely acid-fast but the rods are not. Jensen (1934) regards *C. equi* as being identical with four cultures of soil bacteria isolated in Australia and thinks the species is a widespread soil saprophyte which has acquired pathogenic properties.

Since *C. fascians* was first shown to be the cause of leafy galls and fasciations on chrysanthemum, sweet-pea and other plants, many isolation plates have been made from a large variety of abnormal plant growths and examined for the presence of *C. fascians* by the author and her colleagues. On the plates bacterial colonies, identical in morphological and physiological characteristics with *C. fascians* but completely non-pathogenic, have frequently been encountered. Some of these colonies were the same yellow colour as the pathogenic strains, others were pink. In some cases in which the origin of the abnormal growth was in doubt (e.g. in 'dwarfing disease' of Polyanthus) all the *C. fascians* type of colonies which developed were avirulent. Also, occasionally, a few of these non-pathogenic colonies have appeared on isolation plates made from the surface of healthy plants. It seems probable that the pathogenic *C. fascians* strains have been derived from saprophytic soil diphtheroids (Bisset & Moore's *Jensenia*) which, under certain unknown conditions have acquired the faculty of producing abnormal growth and gall formation in plants. Up to the present, all attempts to induce the saprophytic species to become pathogenic have failed.

Thus we have two pathogenic species, *C. fascians* on plants and *C. equi* on animals, both of which may have been derived from saprophytic soil organisms. In both species the morphology of the cell varies with the medium on which it is grown and—a most distinctive feature—both exhibit acid-fastness when grown on special media.

The close morphological relationship between Corynebacteria, Mycobacteria and Actinomycetes is indicated in all the early classifications, and Jensen (1931) placed the two former genera with the genus *Proactinomyces* in one family, the Proactinomycetaceae. In the fifth edition of Bergey's *Manual of Determinative Bacteriology* (Bergey, 1939) the order Actinomycetales comprised two families, the Mycobacteraceae (for *Corynebacterium* and *Mycobacterium*) and the Actinomycetaceae, one genus of which, *Proactinomyces* (Jensen), was composed entirely of soil species which generally formed short filaments in the early stages of growth but quickly fragmented into rods and coccoid forms. In the sixth edition (Bergey, 1948) however

Corynebacterium has been transferred to the Eubacteriales and the genus *Proactinomyces* is discarded. Bisset & Moore (1949) and Jensen (1953) do not approve of this new classification. Jensen says: 'while the typical representatives of *Corynebacterium*, *Mycobacterium* and *Nocardia* are easy enough to recognize on the basis of morphology, staining reactions and type of carbon metabolism, the genera are linked by boundary forms of which the position is difficult or impossible to define because the distinctive features overlap.'

Corynebacterium fascians is a good example of one of these boundary forms.

I wish to thank Dr S. E. Jacobs for his interest and advice.

REFERENCES

Bergey, D. H. (1939). *Manual of determinative bacteriology*. Ed. 5. London: Baillière, Tindall and Cox.

Bergey, D. H. (1948). *Manual of determinative bacteriology*. Ed. 6. London: Baillière, Tindall and Cox.

Bisset, K. A. & Moore, F. W. (1949). The relationship of certain branched bacterial genera. *J. gen. Microbiol.* **3**, 387–391.

Brooks, R. F. & Hucker, G. J. (1944). A study of certain members of the genus *Corynebacterium*. *J. Bact.* **48**, 295–312.

Conn, H. J. (1947). A protest against the misuse of the generic name *Corynebacterium*. *J. Bact.* **54**, 10.

Conn, H. J. & Dimmick, I. (1947). Soil bacteria similar in morphology to *Mycobacterium* and *Corynebacterium*. *J. Bact.* **54**, 291–303.

Demaree, J. B. & Smith, N. R. (1952). *Nocardia vaccinii* n.sp. causing galls on blueberry plants. *Phytopathology*, **42**, 249–252.

Dowson, W. J. (1942). On the generic name of the Gram-positive bacterial plant pathogens. *Trans. Brit. mycol. Soc.* **25**, 311–314.

Jensen, H. L. (1931). Contribution to our knowledge of the Actinomycetes. II. The definition and subdivision of the genus *Actinomyces* with a preliminary account of Australian soil actinomycetes. *Proc. Linn. Soc. N.S.W.* **56**, 345–370.

Jensen, H. L. (1934). Studies on saprophytic mycobacteria and Corynebacteria. *Proc. Linn. Soc. N.S.W.* **59**, 19–61.

Jensen, H. L. (1953). The genus *Nocardia* (or *Proactinomyces*) and its separation from other Actinomycetales, with some reflections on the phylogeny of the Actinomycetes. *VIth Int. Congr. Microbiol. Rome, Actinomycetales Symposium*, 69–88.

McClung, N. M. (1950). Morphological studies in the genus *Nocardia*. II. Cytological studies. *J. Bact.* **59**, 589–602.

Mohanty, U. (1951). *Corynebacterium fascians* (Tilford) Dowson; its morphology, physiology, nutrition and taxonomic position. *Trans. Brit. mycol. Soc.* **34**, 23–34.

Morton, H. E. (1940). *Corynebacterium diphtheriae*. II. Observations and dissociative studies—the potentialities of the species. *J. Bact.* **40**, 755–814.

Starr, M. P. & Weiss, J. E. (1943). Growth of phytopathogenic bacteria in a synthetic asparagine medium. *Phytopathology*, **33**, 314–318.

Yegian, D. & Baisden, L. (1942). Factors affecting the beading of the tubercle bacillus stained by the Ziehl-Neelsen technique. *J. Bact.* **44**, 667–672.

EXPLANATION OF PLATE 1

Fig. 1. *Corynebacterium fascians*, str. 520. Branching filament stained with methyl violet. × 1500.

Fig. 2. *C. fascians*, str. 505 stained by Ziehl-Neelsen's acid-fast stain with the addition of nigrosine on the lower half. Short cells each contain two acid-fast bodies. The characteristic 'palisade' grouping of cells is also shown. × 1500.

Fig. 3. *C. fascians*, str. 503. Streptococcal-like appearance of acid-fast bodies in films stained by the acid-fast method without a nigrosine background. × 1500.

Fig. 4. As fig. 3 with addition of nigrosine.

Fig. 5. *C. fascians*, str. 519. Filaments showing two types of acid-fast material, (*a*) strongly acid-fast, deeply stained bodies and (*b*) lightly stained granules. × 1500.

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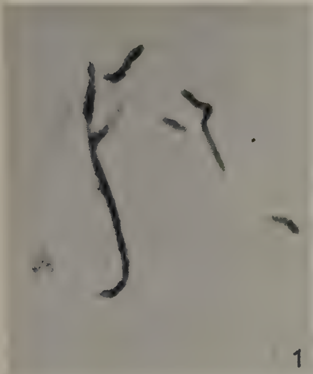

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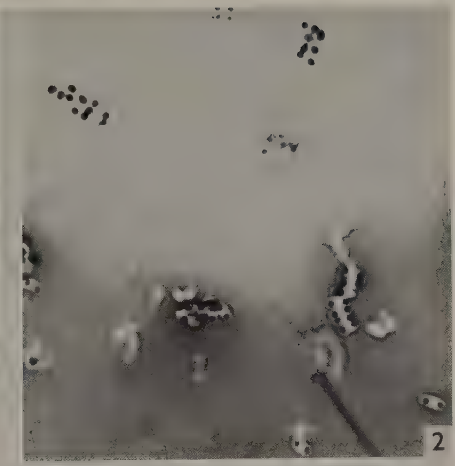

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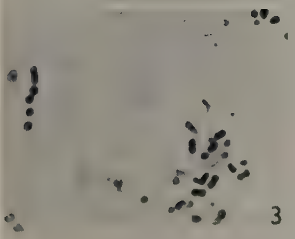

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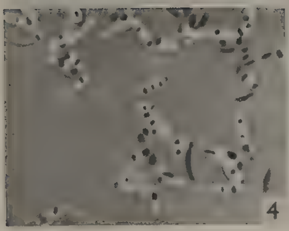

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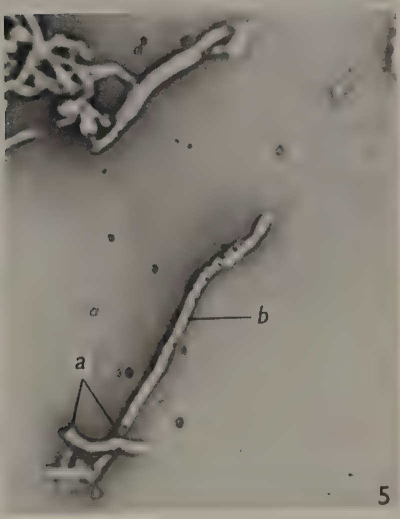

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FAIRY RING KINETICS

By A. F. PARKER-RHODES
University of Cambridge

(With 2 Text-figures)

The behaviour of a system of fairy rings, and in particular its condition on attaining a steady state, is studied theoretically with the help of a simple geometrical model. From the properties of this model, the proportion of the ground occupied by the mycelium is calculated and shown graphically as a function of the growth rate for different values of the reproduction rate. The consequences of interaction between fairy rings of different species, and the factors affecting the mean lifetime of the rings, are also considered.

Conclusions are drawn from these relationships regarding the adaptation of larger fungi for life in various habitats. The ring habit is very inefficient and the communities to which it gives rise very open; these conditions are likely to promote the evolution of a number of species of nearly identical ecological requirements. It is found that there is a minimum area for any species in which it can produce a stable ring system; the possible means by which Basidiomycetes can compete successfully in smaller areas are discussed.

These points, though somewhat conjectural, touch many subjects of general biological and ecological significance, and may be of value in suggesting further lines of fruitful experimental inquiry.

1. Introduction

We are so familiar with the phenomenon of fairy rings that we do not always appreciate what interesting and peculiar problems of ecology and biology they raise. Their practical importance, though it has never been accurately assessed, is no doubt considerable, seeing that with many species their effect is marked both on the growth and on the ecological succession of the herb layer, often agricultural grassland. It is therefore desirable to know as much as possible of the properties belonging to this habit, and of the kinds of fungus communities to which it can give rise.

A large grass field carrying a system of fairy rings can be visualized as like a pond in a light shower of rain. Each ring grows outwards at a constant (or irregularly fluctuating) rate, and new rings are added to the system with approximately constant frequency. When rings of the same species meet, then, unlike ring waves on water, their intersected portions are obliterated; but as between different species either or both may survive the intersection. The important thing is that no ring ever remains still, but only stops growing when it dies. However, all these processes take place with extreme slowness; in the rain-drop analogy the rings grow at a few feet per second, and new ones appear at the order of ten per square foot per second, whereas fairy rings grow at a few feet per year and appear less often than ten per hundred acres per year.

The following are the main points of interest regarding fairy ring systems which are open to theoretical generalizations. First, the proportion of the ground occupied by the mycelium; secondly, the factors affecting interspecific competition in mixed ring systems; thirdly, the age distribution of individual rings. I propose to examine each of these questions with the help of mathematical analysis; fortunately it is possible to construct a very good geometrical model of such a system, from which its behaviour can be deduced with reasonable accuracy, using only elementary calculus.

2. Geometry of the system

It is assumed that the system consists of mycelia growing at a uniform and constant rate ρ, on a uniform and constant plane field of area A. If the rate is not in fact constant, but if the variations are common to the whole system, the effect is simply that of a modified time scale, so that no real loss of realism is involved here. The system has thus two spatial dimensions; we can therefore represent the age of the system as a third spatial dimension, and all the properties of the system will be inherent in the resulting solid figure. In this figure each ring is represented by a right cone; for simplicity the whole system will be regarded as enclosed in another right cone. Since we shall be largely concerned with steady state conditions, this bounding cone can be regarded as very large and the significant part of the system as concentrated near its axis. Two intersecting (and mutually obliterating) rings are represented by the *exposed* surface of two intersecting cones.

In relation to a given ring (which I shall call the *preposite*) any other ring will be called an *impingent* if it has intersected or is destined to intersect the preposite. Any ring which wholly encloses the preposite will be called an *amplectant*. If a ring A has an amplectant A' and a second ring B has no amplectant not common to both rings, then B will impinge on A', their intersected portions will disappear, and B will thus never impinge on A. It is thus easy to see that the condition that two rings should be mutually impingent is that the number of amplectants of each which are not also amplectants of the other should be equal. In this sentence we must allow compound rings, i.e. those formed by previous intersection of two or more, to count as single amplectants; however, it will in due course appear that the probability of any preposite having a compound amplectant both of whose elements are also amplectants is so small that the results will be scarcely unchanged if we count only single rings as amplectants, which greatly simplifies the mathematics.

In the cone model, the condition that a ring should be an amplectant is evidently that the vertex of its cone should lie within the anticone of its preposite (see Fig. 1, where P represents the preposite, and $B+D$ constitutes its anticone). The condition that such an amplectant should not be also an amplectant of another ring Q is therefore that it should not lie in the intersection of the two anticones (i.e. not in D in Fig. 1). The mean numbers of amplectants peculiar to each ring are therefore proportional to the volumes B and C respectively (the number of vertices per unit volume, representing the rate of initiation of rings, being constant). For the case where the bounding cone is very large it can be shown from elementary geometry that the approximate relations hold:

$$C=\tfrac{1}{2}\pi\rho^2T^2(t''-t'+t), \qquad (1a)$$

$$B=\tfrac{1}{2}\pi\rho^2T^2(t''+t'-t), \qquad (1b)$$

where t represents the age (i.e. height of cone) of the preposite P, t' is the age of ring Q, $t''\rho$ the distance between their centres, and $T\rho$ the mean radius of the field. If σ is the number of new rings per unit area per unit time the number of vertices within the prescribed volumes are therefore σB and σC respectively. It can be shown from the conditions postulated above that the probability that P and Q are mutually impingent is

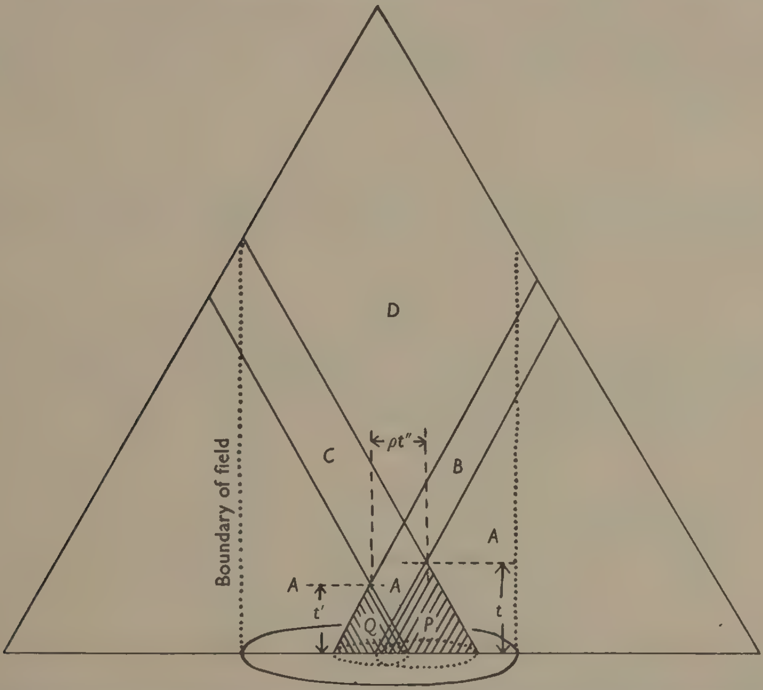

Fig. 1. The main points of the geometrical model. The diagram is not drawn to scale, in that, with the proportions shown, the approximations used in the calculations would scarcely be acceptable.

$$p(t,t',t'')=\exp[-\sigma(B+C)]\sum_{i=0}^{\infty}\frac{(\sigma BC)^i}{i!^2}. \qquad (2)$$

3. RATE OF GROWTH OF IMPINGENT GROUPS

Let us define an *impingent group* as consisting of such rings as are mutually impingent. It is evident from this definition that all the rings in the system can be classified into a set of mutually exclusive impingent groups. Every point in the field will be traversed once and once only by the boundary of every impingent group. The mean interval between one such transit and the next may be called the *generation time* of the groups.

Suppose now that the effective radial width of the rings is r, and that the generation time is τ. Then, out of a period of time τ each point in the field will spend a mean time r/ρ within the mycelial area, so that the proportion of the ground occupied by the latter is

$$x = r/\rho\tau. \tag{3}$$

Now the volume contained within the compound figure of intersecting cones which represents an impingent group up to the moment of origin of its successor must be that containing one vertex. The height of this figure is on the average τ. If then we denote the area at time t of the group by $a'(t)$, we have

$$1 \doteq \sigma \int_0^\tau (a'(t) - a'(0))\,dt. \tag{4a}$$

Since the rings will in general overlap, the sum of the areas of all the rings a will be in general greater than a', and elementary sampling theory shows that on the average

$$a' = A(1 - e^{-a/A}), \tag{4b}$$

where A is the area of the whole field. To find τ, and hence x, we have therefore to evaluate this integral. The working of this problem will be found in the Appendix. The result is that equation (4a) reduces approximately (near the centre of a large field, with known orders of magnitude of the quantities involved) to

$$1 \simeq \sigma \int_0^\tau A \left\{ \exp\left[-\frac{64\pi^2\rho^4}{A^6\sigma^4} \right] - \exp\left[-A^2\sigma^2 t^2 \left(\frac{\pi\rho^2}{A^3\sigma^2} + \frac{8\pi^2\rho^4}{A^6\sigma^4} \right) \right] \right\} dt, \tag{5}$$

from which we can derive the equation for τ in the form

$$\tau \simeq e^{64c^2} \left\{ \frac{1}{A\sigma} + \frac{(-\frac{1}{2})!\,[A^2\sigma^2\tau^2(c+8c^2)]}{2A\sigma\sqrt{(c+8c^2)}} \right\}, \tag{6}$$

where $$c = \pi\rho^2/A^3\sigma^2.$$

This equation can be solved for τ in terms of A, ρ and σ, by successive approximation (the incomplete factorial can be obtained from tables of the normal probability integral). The results are shown graphically in Fig. 2, where the values of x are plotted against the growth rate ρ for different values of (a) σ, and (b) the product $\rho^2\sigma$.

4. INTERSPECIFIC COMPETITION

The data exhibited in Fig. 2 can be used to assess the theoretical conditions governing the competition between species of ring-forming fungi. Three separate cases have to be considered, according to what happens when rings of different species impinge. In the first case, which I shall call *indifference*, both rings continue to grow and fructify after intersecting; in the second case, of *bilateral extinction*, both rings are obliterated in the intersecting region, as if they were of the same species; in the third case there is *unilateral extinction*, one ring being unaffected while the other is eliminated.

In unilateral extinction the species eliminated can maintain no equilibrium at all, and its occupation of the territory is dependent on the continued arrival of propagula from elsewhere (unless the reproduction rate is so great that even a very small population can produce sufficient propagula to colonize the transient vacancies). In general, however, the conditions are so unfavourable that such species will exist only in immature ring systems, or where their successful competitors are excluded by other factors.

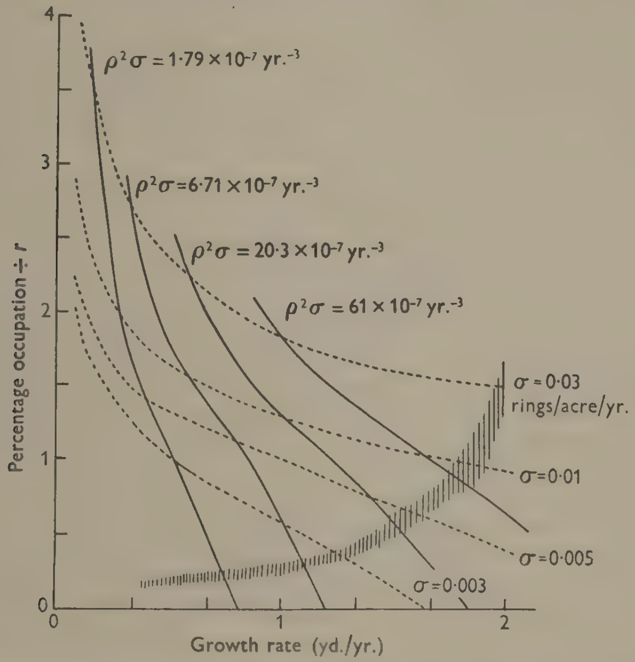

Fig. 2. The curves are drawn for a field of 10 acres; for larger fields the part of the diagram above the shaded zone is unchanged, except for the labelling of the contours, which will be governed by $A\sigma = \text{const.}$ For smaller fields the diagram rapidly becomes inapplicable as explained in the text. The quantity r is the effective width of the active zone of the rings, whose value will depend *inter alia* on the definition of 'cover' which one works to.

In cases of indifference, the two ring systems will simply co-exist; they will attain a steady state in which both have a finite proportional occupation, whatever the relation between their parameters, and the only index of relative success will be their net reproductive efficiency per unit area.

In bilateral extinction (the most interesting case, since it will cover very closely related species), the one with the larger growth rate will eventually eliminate the other, though the time required will be very long unless the difference is large; we assume equal values of σ. (This may be seen by considering the intersection of two cones with parallel axes but different vertical angles; at a sufficient distance from the vertices the cone with the larger angle will wholly enclose the other.) Likewise, if both have the same growth rate ρ, but different reproduction rates σ, the one with the greater σ will after sufficient time (again likely to be very long) eliminate the other. Application of the method of dimensions shows that the condition that both species should have a finite occupation density at the steady state is that their values of the product $\rho^2\sigma$ should be equal. It follows that of two bilaterally competing species, the one with higher $\rho^2\sigma$ will always tend to oust the one with the lower value. Thus the eventual survivors of such a competing group will tend to have the same $\rho^2\sigma$, and this the highest possible for them. Of two such, the one with higher σ and lower ρ will be able to achieve the higher proportional occupation of the ground, though this is not necessarily decisive for survival.

Inspection of Fig. 2 will now show that there will be a general tendency towards more rapid reproduction of rings and slower growth rate. Since the ring habit is itself dependent on the exhaustion or poisoning of recently occupied ground, there is likely to be a definite lower limit to the growth rate, and there is also an upper limit to the rate of reproduction. Thus we may expect a fairly definite clustering of the values of steady-state occupation about the optimum thus determined. Consideration of the order of magnitude of the actual rates observed in nature leads us to expect this to be of the order of 0·05, i.e. 5 % of the ground occupied, based on the arbitrary assumption of 2 yards for the effective thickness of the rings. Data which I have obtained on the ecology of fungi in Skokholm Island, making this same assumption, bear out this expectation fairly well (Parker-Rhodes, 1953). Any other figure for the r of equation (3) would be equally consistent if applied to the same data, though the 'observed' and 'calculated' percentages would both differ from the figures given here. Fig. 2 also shows that, despite the general tendency towards slow growth, species subject to bilateral extinction may tend to a higher growth rate, owing to the steep slope of the $\rho^2\sigma$-contours.

There is unfortunately little direct experimental evidence to test these conclusions. There are, indeed, only a few references in the literature to what happens when two rings of different species meet. Shantz & Piemeisel (1917) report that *Agaricus campestris* is extinguished unilaterally by *Calvatia cyathiformis*, but this is the only recorded case of unilateral extinction known to me, though there must be others awaiting discovery. Indifference is no doubt much the commonest condition, but again specific confirmation is lacking; one proven instance has been given by myself (Parker-Rhodes, 1950, pl. 8) with *Lactarius rufus* and *Collybia maculata*. Bilateral extinction is presumably the rule with the most closely related species, but it may well occur in other cases also.

To some extent the long-term conclusions of this section are open to question, since, as we shall see in §7, the time required to reach a steady state may in some circumstances, especially before the coming of man broke up the primeval grasslands of temperate climates, be so long that species may become adapted specifically to existence in juvenile ring systems. But in default of further evidence speculation is idle.

5. Non-uniform fields

In the foregoing discussion it has been assumed that the field of the ring system was large, that is to say, boundary effects were neglected. Exact treatment of this problem is difficult, and the mathematical models required can never be very realistic; it is sufficient therefore to consider the matter qualitatively. Supposing the field to be bounded by ground uninhabitable by the mycelium, the percentage occupation will be less at the edge than near the centre, because there mycelium can only arrive from one side instead of from all round. In the ideal case of an infinite straight-edge boundary, the reduction will be exactly half; it will become negligible at distances from the boundary of the order of the distance covered by the growing mycelium in the generation time τ of an impingent system.

Similarly, any limited region of uninhabitable territory within a large field, such as an outcrop of rock, will be surrounded by a zone of similar width in which the mean proportional occupation will be reduced, though by less than half. Conversely, a narrow promontory from the field, surrounded on most sides by uninhabitable ground, will support a mean occupation of less than half the general mean.

If the field is appreciably smaller than the 10 acres assumed in Fig. 2, the mean occupation will be rather drastically curtailed. Other factors being equal, the occupation density is proportional to $A.\exp[-kA^{-6}]$. With the optimum plausible figures of $\sigma = 0{\cdot}1$ ring per acre per year and $\rho = 6$ in. per year, k is approximately 100, and the exponential factor falls from about $\frac{1}{2}$ to virtually zero as A falls from 2 to 1 acre; for less favourable σ and ρ the vanishing will occur for larger areas. It follows that fairy ring systems cannot permanently exist on areas appreciably less than 10 acres. It obviously does not imply that rings cannot occur on such areas, as everyone who has a lawn must know, but that such rings will be very infrequent when (if ever) a steady state is reached. These small areas can therefore play only a negligible part in the ecology of long lived ring-formers such as we have been considering.

6. Factors affecting the effective reproduction rate

It is evident that one of the most important factors for ring-forming fungi is the effective rate of reproduction of new rings. Any means, therefore, by which this could be increased, may be of advantage to the species. Two possibilities may be considered: homothallism, and spontaneous breaching.

Obviously, if the successful germination of at least two spores of appropriate 'sex' is necessary for the establishment of a new fruiting ring, the rate of such new establishments will be less than if a single germination were effective. Since there is no reason to suspect that homothallism necessarily carries with it any important loss of growth rate, a homothallic race will usually have a larger value of $\rho^2\sigma$ than heterothallic races of the same species which will react with it in bilateral extinction. We must therefore expect to find few cases of both habits co-existing in obligately ring-forming species, unless perhaps geographical or ecological isolation mitigates the relationship. In particular, if a homothallic strain of any heterothallic species appears, it would be expected to eliminate, in time, all the latter, unless some are saved by effective barriers. This would of course be a long-term disadvantage to the species affected, by curtailing its capacity for variation; we might therefore expect that fairy ring fungi would be exceptionally resistant to relapsing into homothallism, except in cases where the homothallism is facultative and rarely exercised. This exception may apply to gasteromycetes, such as the Lycoperdacei, several species of which are reported as homothallic, since their spores are normally distributed in relatively dense clouds so that most rings will be in practice the product of multiple germination whatever a single spore may be able to accomplish in a Petri dish.

Turning now to spontaneous breaching, let us suppose that by some means (say a lethal gene locally homozygous through caryallaxis with a new germling) a length of the perimeter of a growing ring is abolished. The subsequent growth of such a ring should ideally proceed with two incurving horns which, when they meet, will reconstitute the closed perimeter and at the same time abstrict a ϒ-shaped portion from which a new ring could grow inside the parent. Such a secondary ring will belong to the next impingent group after that of its primary; the points of origin of secondaries will lie at a more or less definite distance within the effective perimeter of the primary. The effect of such spontaneous breaches on the growth of an impingent system requires a little consideration.

In the geometrical model (Fig. 1) all secondary cones of a given impingent group, having a present radius $\rho t'$, have their vertices on a circle at a height t' and of radius $\rho(T+t-t'-t_0)$ (where $T+t$ is the age of the impingent group to which the secondaries belong, and t_0 is the delay occupied in the process described above). Evidently the aggregate area b contributed to the system by the secondaries will be

$$b=\int_0^{T+t-t_0} 2\pi^2\rho^3\lambda_0 . t'^2(T+t-t'-t_0) . dt'$$

$$=\tfrac{1}{6}\pi^2\rho^3\lambda_0\{t^4+4T''t^3+6T''^2t^2+4T''^3t+T''^4\}, \tag{7}$$

where
$$T''=T-t_0\simeq T$$

and λ_0 is the rate of breaching. This expression will combine additively with the a of §3; it will be seen that the coefficient of t in this polynomial will be of comparable order with that in a (see equation (13) in the Appendix), and therefore negligible in comparison with that of t^2, only if

$$\lambda_0\sim 63\pi^{\frac{3}{2}}\rho^4/A^{\frac{5}{2}}(A\sigma)^3, \tag{8}$$

which, with $A=5\times 10^4$, $A\sigma=10^{-1}$, $\rho=1$, makes $\lambda_0\sim 10^{-6}$, in which case the effect of the breaching on the effective reproduction rate would be small; if therefore the effect is to be appreciable, the new polynomial outweighs the old, and its dominant term being in t, not in t^2, the equation for τ will become

$$\tau=\left\{\frac{1}{A\sigma'}+\frac{1-\exp[-2\pi^{\frac{1}{2}}\lambda_0 A^{\frac{1}{2}}\tau]}{2\pi^{\frac{1}{2}}\lambda_0 A^{\frac{1}{2}}}\right\}e^{A\lambda_0/2\rho}. \tag{9}$$

From this it is apparent that except for very small values of λ_0 the effect of spontaneous breaching followed by formation of secondary rings will be to reduce the proportional occupation of the ground at the steady state, the effect increasing rapidly with the area of the field; in fact, under these conditions, the extra rings increase the sweeping effect more effectively than they add to the number of fructifications. If we take the case where $\lambda_0 \sim 10^{-6}$, so that equation (6) is still approximately correct, the number of secondaries added within a given impingent group during the period τ will be $3\lambda_0\sqrt{(1+\rho^2)}/\rho$; with $x = 0{\cdot}05$ and $\rho \sim 1$ yd. per year, this quantity is $\sim 10^{-3}$ of σ, whereas for $\rho \sim 10^{-1}$ yd. per year, the two quantities are comparable. Thus spontaneous breaching could only be an appreciable factor in raising the proportion of occupied ground if the growth rate were very small and the field area not much in excess of 10 acres.

7. LONGEVITY OF FAIRY RINGS

It is commonly assumed that ring-forming fungi are among the most long-lived of all organisms. If we regard any secondaries which may be formed as above as continuations of their primaries, this may well be correct, but it is more natural to regard this as a case of vegetative reproduction, and to define longevity in terms of the geometrically continuous perimeter.

The problem can be approached by the method of dimensions. There are three parameters which may be considered relevant; the growth rate ρ, the reproduction rate σ, and the surface density of surviving rings; this last may be replaced, if we leave out the total number of rings (which is of course a dimensionless quantity), by the field area A, though care will be required in its interpretation. The first of these parameters can, paradoxically, be ruled out as a mere scale factor; for although an increase in ρ will reduce the time needed for a ring to reach a given size, it will simultaneously increase the size reached by the ring when its first successor appears, which, as is evident from §3, is what really determines the time scale of the system. It follows that, in general, the mean life is

$$\bar{t} = k/A\sigma, \tag{10}$$

where k is a numerical constant which we shall evaluate.

At first sight this equation appears paradoxical, as it implies that rings tend to live longer in a small field than in a large one. We must remember, however, that steady-state conditions are implied, and that the total number of rings does not appear in the equation. If we suppose that a given number of rings in a small field are spread out more widely, they will obviously take longer to impinge and so live longer; but if they were formerly in a steady state they will be so no longer, and the number of rings will increase until a new steady state is reached, which will not occur until equation (10) is again satisfied. Actually (though I have avoided diachronic considerations in this paper) it will take so long to reach a steady state in a large field of say more than 100 acres that under such conditions the foregoing analysis is scarcely relevant. Such large areas of uniform conditions for ring growth are rarely encountered in the presence of intensive human settlement, and are therefore of little economic importance; but unfortunately the chief quantitative work on this question up to date, that of Shantz & Piemeisel (1917), was done in such conditions in the American prairies. Their data must therefore be taken as referring to a relatively juvenile state of the ring system. In Western Europe the rings in such aged or undisturbed vegetation as remains and is of economic interest (e.g. so-called permanent grass, marginal grazing lands, etc.) will usually be in nearly steady-state conditions.

We may rewrite equation (10) less paradoxically, by taking account of the actual number N of surviving rings in the field, and writing $A = N/\nu$ where ν is the surface density of the rings. This last at the steady state can depend only on ρ and σ, and the method of dimensions gives the result $\nu = h\sigma^{\frac{2}{3}}\rho^{-\frac{2}{3}}$; substituting this into equation (10) then gives us

$$\bar{t} = hk/N\rho^{\frac{2}{3}}\sigma^{\frac{1}{3}}, \tag{11}$$

which shows the virtual dependence of $\bar{t}$ on ρ on the rather artificial assumption that N is fixed.

The constant k can be evaluated from the geometrical consideration that the product $A\sigma\bar{t}$ is the average ring's share of $A\sigma T$ (ρT being the mean radius of the field) and that this is 3 times the total number of rings present; since at the steady state each ring must have on the average exactly one successor, it follows that $A\sigma\bar{t} = k = 3$. The constant h in equation (11) can be found from the fact that, if we consider a single ring only, itself constituting the whole of its impingent group, its lifetime must be in general equal to the generation time of the latter. Thus, for $N = 1$ equation (11) should reduce approximately to (6), whence $h = 3^{-\frac{2}{3}} = 0{\cdot}481$ approx.

The equation $\bar{t} = 3/A\sigma$ shows now that the order of magnitude of the mean lifetime of rings at a steady state should usually be from 30 to 300 years. This is broadly speaking borne out by such observations as are available. In those cases, such as very extensive fields, where steady states are not reached, lifetimes may be much greater than this, and under these conditions the supposed great longevity of fairy rings may be true. But in situations here regarded as typical they are likely to be less long lived than many species of forest trees, for example, and so, probably, less also than their mycorrhizal fungi.

Always up to now we have made the assumption that there is no intrinsic cause of death other than the mutual interference of the rings. This, obviously, is not strictly true. Though the observations of Shantz & Piemeisel indicate that some rings can live as long as the above figures predict, their maps also make clear that these old rings are always fragmentary, and the breaches which appear in them are not normally filled in the manner envisaged above, but tend to be permanent; moreover, many of their figures can only be explained on the assumption that half-rings and smaller fragments may persist for a long time without reforming themselves into complete perimeters. There are also reports in the literature suggesting the simultaneous death of a whole ring at a relatively early age; such an occurrence is recorded for example for *Aspropaxillus giganteus* by Rosenvinge (1933). If the average lifetime of a ring, in view of these hazards, is nevertheless of the same order of magnitude as the $\bar{t}$ calculated above, our conclusions will not be much affected. But if their effect is to reduce the average lifetime appreciably below this, the effect will not be negligible. If the expected proportion of compound rings is small, attainment of the steady state will be delayed, and the proportional occupation achieved by the system will be reduced; but if there would be a substantial proportion of compound rings if they survived long enough, premature decease will have the effect of a reduction of growth rate, and so increase occupation percentages. The modification required in Fig. 2 is unlikely to be appreciable, except for the lowest reproduction rates, whose unfavourable effects on occupation will be less than indicated in the graphs.

One important effect of premature decease may be its effects in interspecific competition with unilateral extinction; for it may enable the weaker species to survive indefinitely, if this should have the advantage in length of life. There is, however, no evidence on this point.

8. Discussion

One general conclusion which emerges from this analysis is that no species of ring-forming fungus can attain an occupation of more than a few per cent of the available ground (excluding ground occupied in the sense of being made uninhabitable by the species, the extent of which is in most cases quite unknown). This is therefore a habit of very low ecological efficiency, and it can only exist at all, one must suppose, on account of some biological impossibility of colonizing those habitats where it is best exemplified in any other manner. That such low density, and consequent open structure of the fungus communities, is a fact, is demonstrated by the results I have obtained on Skokholm Island (Parker-Rhodes, 1953). The work of Shantz and Piemeisel, as I have already mentioned, cannot be cited here since it does not refer to a steady state, whereas on Skokholm the available fields, being relatively small (up to about 25 acres), are probably already in this condition. There are in fact rather few obvious rings of any species there, which is what one would expect of a mature system. It is interesting also that the one species for which there are few ecological barriers on the island, and therefore disposes of a field of well over 100 acres, is *Lepiota procera*, which exhibits a ring system of the 'juvenile' type, similar to those described by Shantz and Piemeisel.

One must conclude, I think, that there is a fundamental property in the physiology of fungi, at least of the larger Basidiomycetes, which makes impossible the continued growth of mycelium in the same body of medium. If that were so, the only non-ring forming species would be those growing in habitats where the annual access of organic matter is sufficient for the year's needs of the fungus; here, continued growth of the fungus would be possible by successive recolonization of the annual layers without lateral spread of mycelium. Such conditions evidently obtain best in forest communities, but are neither universal in nor limited to these; Pteridieta, for example, provide a fairly considerable rate of access, as do Sphagneta and many other hygrophytic associations. In these, one may suppose, the ring habit is obligatory only on the species affecting the deepest layers of the humus, and will be a considerable handicap to the rest. There are indeed some species which often or usually form rings even in woodland leaf-humus, such as *Craterellus tubaeformis*, but the growth of these may well depend merely on local variations in the rate of access of organic material, which may be considerable.

On this hypothesis, the ring habit would only be typical in those plant associations having a low rate of access, such as poor grassland and other low-growing vegetation. This is, of course, borne out by general experience. There remains, however, the problem of those habitats which combine a low rate of access with small areal extent, that is, with frequent intermission of the characteristic flora; such, for example, are many mountain and coastal habitats where the vegetation is much dissected by rocks, clearings and small open spaces in woods on thin soils, sand dunes, burnt ground, and most ruderal habitats. Such places are attractive to relatively few species, but intensive study generally reveals the presence of more fungi than they are usually credited with. We have seen that such small areas are unlikely to be adequate for ring-formers of the character we have been discussing, and this prompts the question of what adaptations would be needed to enable a Basidiomycete successfully to compete for them. One possibility would be to reduce the linear scale of their operations, producing scanty and restricted mycelia, small growth rates, and small basidiocarps; such a species could maintain a stable ring system in a smaller area than more expansive forms. Such a one is *Crinipellis stipitarius*, one of the commonest species of such habitats. Conversely, however, slow growth rates would be a considerable disadvantage in open competition with typical ring-fungi.

Another adaptation serving the same end would be an increased reproduction rate, which would make possible the rapid replacement of rings extinguished by having reached the boundaries of the field. This raises the question, why is the actual reproduction rate of typical ring fungi so extremely low; that it is so seems borne out by all observers (the data of Shantz and Piemeisel are relevant here). The reason is not far to seek: if the normal course of events were that a single spore should germinate to establish a uninucleate mycelium, the great majority of rings in a heterothallic species would remain sterile until their first impingement. It can, however, be shown from equation (6) that the majority of rings in a stable system with optimal parameters will be thus virgin (the compound rings are in a majority only in the bottom region of Fig. 2). Therefore a species with this habit would be supporting a large haploid population, itself a retarding factor on evolution, without the benefit of much gain in reproductive capacity. Thus, as in the analogous case of homothallism, there will be a contradiction between the short-term advantage of reproduction rate and the long-term advantage of evolutionary potential; the latter is best served by allowing only a very brief life to the uninucleate mycelium, so that at least two compatible spores must germinate together in order that a new ring be initiated, and this we may take to be the general rule among ring-fungi.

These considerations do not apply in the case of species which affect small fields, such as we have seen to be incapable of supporting stable ring systems; in these habitats the increased reproduction rate made possible by the presence of monospore uninucleate rings awaiting diploidization through a second germination may outweigh other considerations. This is perhaps the condition of such comparatively large species characteristic of ruderal habitats as *Volvaria speciosa*, which sometimes appears, seemingly for the first time, in large rings. Though such species are also found in ordinary open grassland, they may depend for ultimate survival on their more circumscribed retreats.

The most obvious remedy for a low reproduction rate would be increased spore production. In this the Lycoperdacei excel, and we might therefore expect to find these fungi especially commonly in restricted habitats; but in fact they are almost invariably typical large-field ringformers. Perhaps, however, a high rate of reproduction may enable such a species of *Calvatia cyathiformis* to survive in juvenile ring systems in the face of unilateral extinction.

Ecologically, the main conclusion from the analysis is that the communities of Basidiomycetes in poor grassland associations will be in general very open, whereas those in most associations with a rate of access of organic matter sufficient to support non-ring-forming mycelia are closed, at least when they have reached a steady state. In such open communities direct interspecific competition, other than that arising from bilateral or unilateral extinction, will be reduced to a minimum. Both these forms of interaction are certainly less frequent, probably much less so, than indifference. This opens an opportunity for the evolution of a large number of species whose niches are differentiated from each other only by such mutual adaptations as are necessary to avoid hostile interactions; for such a set of species can exploit the substrate more effectively than any one of them, not through complementary ecological specialization but in spite of identical requirements. It is of course almost impossible to adduce evidence for so negative a thesis; but it is at least significant that the number of species of Basidiomycetes in such grassland is often much greater than that of the plants, notwithstanding the simple structure and high uniformity of the habitat. In view of this conclusion it is perhaps unnecessary to expend our ingenuity in trying to apply Gauss's principle to these fungi.

APPENDIX

Derivation of the equation (5)

The area of the preposite ring is $\pi\rho^2 t^2$; that of any other ring of present radius t' is $\pi\rho^2 t'^2$. The number of rings having a radius within the range $\rho(t' \pm \frac{1}{2}\delta t')$ and a central distance from the preposite within the range $\rho(t'' \pm \frac{1}{2}\delta t'')$ is obtained from the element of volume of the cone model in which their vertices lie, and is $2\pi\rho^2\sigma t''\,\delta t'\,\delta t''$. The chance that any one of these is an impingent on the preposite, and therefore the proportion of them which belong to the impingent group, is $p(t, t', t'')$. Therefore their aggregate area is given by the double integral

$$a = 2\pi^2\rho^4\sigma\int_0^T\int_0^{t''+t} t'^2 t'' p(t, t', t'')\,dt'\,dt'' + \pi\rho^2 t^2$$

$$= 2\pi^2\rho^4\sigma\int_0^T t'' e^{-A\sigma t''}\int_0^{t''+t} t'^2\left(1 + \frac{A^2\sigma^2}{4}(t''^2 - \overline{t-t'}^2) + \dots\right)dt'\,dt'' + \pi\rho^2 t^2. \quad (12)$$

Now, the quantity $A\sigma$ is equal to the absolute rate of appearance of rings over the whole field, which is in practice of order less (usually much less) than one per year; we may therefore approximate (near the centre of a large field) by ignoring further terms of the series. Moreover the limit T in the integral over dt'' is the time required by the preposite ring to grow to the size of the whole field, which we may take as infinite. With these approximations the integral can be evaluated as

$$a=\frac{2\pi^2\rho^4}{A^5\sigma^4}\left\{32+21(A\sigma t)+\left(\frac{A^3\sigma^2}{2\pi\rho^2}+4\right)(A\sigma t)^2+\tfrac{5}{6}(A\sigma t)^3-\frac{(A\sigma t)^5}{120}\right\}. \quad (13)$$

This expression can be further simplified, when account is taken of the orders of magnitude of the quantities involved. We know that in general $A \geqslant \sim 10^4$ yd.^{2}, $A\sigma < \sim 10^{-1}$ per year, and $\rho \sim 1$ yd. per year, and it can be shown that with these values the terms in t^3 and t^5 will not exceed 1/20 of that in t^2, which contains 90 % of the whole quantity in all practical cases. In that case the equation reduces to

$$\frac{a}{A} \simeq \frac{64\pi^2\rho^4}{A^6\sigma^4}+\left(\frac{\pi\rho^2}{A^3\sigma^2}+\frac{8\pi^2\rho^4}{A^6\sigma^4}\right)(A\sigma t)^2, \quad (14)$$

which substituted in equation (4) gives (5) of the text.

REFERENCES

PARKER-RHODES, A. F. (1950). *Fungi, friends and foes.* London.

PARKER-RHODES, A. F. (1953). The Basidiomycetes of Skokholm Island. X. Population densities. *New Phytol.* **52**, 273–291.

ROSENVINGE, L. K. (1933). En Hexering i Jaegersborg Dyrehave. *Bot. Tidsskr.* **42**, 217–231.

SHANTZ, H. L. & PIEMEISEL, R. L. (1917). Fungus fairy rings in Eastern Colorado and their effect on vegetation. *J. agric. Res.* **11**, 191–245.

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HYPOGEOUS FUNGI. IV AND V

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(With 2 Text-figures)

Fruit-bodies of a species of *Rhizopogon* collected near Burrington, Somerset, in the autumn of 1953, could not be assigned to any known species of this genus and are here described as *Rhizopogon reticulatus* sp.nov. Other fruit-bodies collected in September 1954 in the New Forest are considered to be those of *R. provincialis* Tul., the first record of this fungus in Britain. A revised key to British species of *Rhizopogon* is given.

Battarina inclusa (Berk. & Br.) Clem. & Shear. was collected on *Tuber puberulum* Berk. & Br. at Wotton-under-Edge, Gloucestershire, 7 November 1953. The spores were found to be sparsely echinulate and comparison with type material showed that previous illustrations of these spores (Berkeley & Broome, 1861; Petch, 1938) are incorrect.

Sepedonium chrysospermum Fr. on *Melanogaster variegatus* Vitt. var. *broomeianus* Berk., collected at Blaise Castle, Bristol, 30 September 1953, is a new British host record. It has since been found also on *Rhizopogon provincialis*.

IV. BRITISH SPECIES OF *RHIZOPOGON*: *RHIZOPOGON RETICULATUS*, A NEW SPECIES FROM BURRINGTON, SOMERSET AND *R. PROVINCIALIS* TUL., A NEW BRITISH RECORD FROM THE NEW FOREST

Rhizopogon reticulatus Hawker sp.nov. (Fig. 1).

Fructificationes globosae vel oblato-sphaeroideae, ca. 2·0 cm. diametro, molles, superficie primo alba dein statu vivo maculis nigris fibrillosis praedita siccitate areolato-rugosa, gleba primo alba dein luteo-olivacea vel viridi-brunnea, tactu nigrescentes; basidia obovato-clavata, 12 × 4·5 μ, 4–7-spora, sterigmatibus fere obsoletis; sporae 7·5–6·5 × 3·0–1·5 μ, fusiformes, biguttulatae, hyalinae, in cumulo pallide olivaceae.

Sub Piceis, Burrington, Somerset, England, 28 September 1953, Herb. Hawker no. 847 Typus.

Fruit-bodies spherical or flattened, *c.* 2·0 cm. diam., soft, at first white, later covered with fine black reticulate hyphae, gleba at first white, later olive-yellow to greenish brown, blackening when bruised, basidia tapering to base, 12 × 4·5 μ, bearing 4–7 almost sessile basidiospores. Spores spindle-shaped, 7·5–6·5 × 3·0–1·5 μ, 2-guttulate, hyaline, light olive-green in the mass.

Under spruce, Burrington, Somerset, 28 September 1953, Herb. Hawker, no. 847, Type material.

Fruit-bodies of a hypogeous Gasteromycete were collected on 28 September 1953 and on 2 December 1953 (nos. 847, 993 and 994 in my collection of hypogeous fungi) in a mixed spruce and larch wood near Burrington, Somerset. Further collections were made in the autumn of 1954. The fruit-bodies were found growing gregariously near the surface of the litter layer in close proximity to the larger spruce roots.

The fruit-bodies were globose to ovoid, or somewhat flattened where they were pressed against the tree roots, and were up to 25 mm. in diameter. Young specimens were whitish, older ones became stained with the red soil and were covered with a network of fine dark strands or fibrils which gave a finely mottled appearance (Fig. 1 A, B). The peridium was thin and rather fragile and showed a tendency to crack in large specimens. Dried material became very wrinkled. The texture of the fresh fruit-bodies was soft and spongy but brittle so that they could be easily broken in pieces. Both peridium and gleba became blackened when bruised or broken. The gleba was white at first, becoming pale olive-yellow and finally dirty greenish brown or even black on exposure to air. The chambers were numerous and minute.

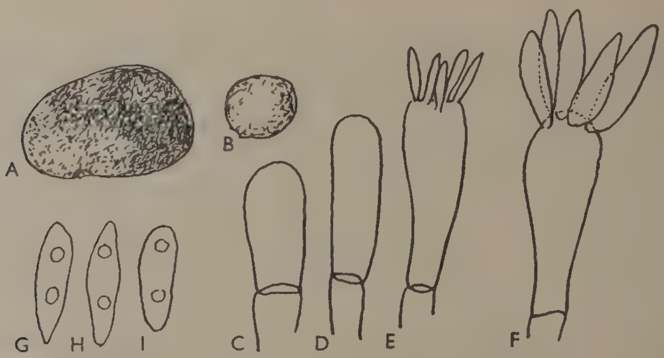

Fig. 1. *Rhizopogon reticulatus.* A, mature fruit-body showing network of dark fibrillar hyphae; B, younger fruit-body. A, B, nat. size. C–F, stages in development of basidium; G–I, mature basidiospores. C–I, × 1800.

The basidia, which were present on even the smallest specimen found, were rounded at the apex and tapered to the base, and averaged 12 μ long × 4·5 μ wide at the widest part near the apex (Fig. 1 C–F). They bore 4–7 almost sessile basidiospores which were irregularly spindle-shaped, 7·5–6·75–6·5 × 3·0–2·17–1·5 μ and contained 2 guttules (Fig. 1 G–I). The spores were transparent but were light olive green in the mass.

These specimens differ from the two previously recorded British species, both of which are usually found under Scots pine. The spores of *Rhizopogon rubescens* are longer and this species lacks the finely reticulate fibres on the peridium. The peridium of *R. luteolus* is tough and leathery and the colour and general appearance of this species is quite different from that of the Burrington material. No published description of any species of *Rhizopogon* could be found to fit the present material which is accordingly described as *R. reticulatus* Hawker sp.nov. (the specific epithet refers to the reticulate fibres on the peridium).

During the autumn foray of the Society in September 1954, numerous fruit-bodies of a species of *Rhizopogon* were collected at Vinney Ridge Enclosure, Knightwood, in the New Forest, Hampshire, under Douglas fir (*Pseudotsuga taxifolia*). These clearly did not belong to either of the previously recorded British species or to *Rhizopogon reticulatus*. The morphology and colour of the fruit-bodies and the spore characters agreed with those of *R. provincialis* Tul. This species has not previously been recorded in Britain and thus the present collection (no. 1077 in my collection) is a new record for this country.

The addition of these two species to the British list makes a new key to the British species of this genus desirable.

Key to the British species of Rhizopogon

1.	Fruit-body reddening when bruised or exposed to the air.	2
	Fruit-body not reddening when bruised or exposed.	3
2.	Peridium thin, spores usually more than 3 μ broad.	**R. rubescens**
	Peridium at least 200 μ thick, spores less than 3 μ broad.	**R. provincialis**
3.	Fruit-body blackening when bruised or exposed to air, peridium covered with fine black reticulate hyphae.	**R. reticulatus**
	Fruit-body not blackening markedly on bruising or exposure, peridium thick, becoming corky or leathery, with coarse black mycelial strands.	**R. luteolus**

It is worth recording here that *R. luteolus*, which in my recent monograph (Hawker, 1954) was said to be recorded only from Scotland, has since been found in quantity by Mr T. J. Wallace in Devonshire (Bicton Common and Haldon Forest).

V. TWO FUNGAL PARASITES OF HYPOGEOUS FRUIT-BODIES: *BATTARINA INCLUSA* ON *TUBER PUBERULUM* AND *SEPEDONIUM CHRYSOSPERMUM* ON *MELANOGASTER VARIEGATUS* VAR. *BROOMEIANUS* AND ON *RHIZOPOGON PROVINCIALIS*.

Battarina inclusa (Berk. & Br.) Clements & Shear

Fruit-bodies of *Tuber puberulum* Berk. & Br. of abnormal size and appearance were collected in a larch plantation at Wotton-under-Edge, Gloucestershire, in November 1953 (no. 968 in my collection). These were unusually large, lobed, and pale in colour for the time of year, when normal fruit-bodies would be tan to mole-grey in colour. When cut through they showed numerous minute yellow-gold spots. These proved to be spherical perithecia containing large numbers of clavate to cylindrical 8-spored asci. The perithecia had no necks, the ascospores were not always uniseriate and were shed into the cavity before reaching maturity (Fig. 2A–C). The parasite agreed with the original description given by Berkeley & Broome (1861) of *Hypocrea inclusa* and with Petch's (1938, p. 247) description of the same fungus under the name of *Battarina inclusa* (Berk. & Br.) Sacc., except that the spores were minutely and sparsely echinulate (Fig. 2D). The spines were very short (1·0 μ) and slender and could only be seen clearly under the highest power objective so that they could easily have been missed by earlier observers. The outer flange or utricle figured by Petch is an optical illusion.

Petch's citation of the fungus is considered by Bisby & Mason (1940) to be incorrect since the name *Battarina* was used by Saccardo as that of a subgenus only. The correct citation is thus *B. inclusa* (Berk. & Br.) Clements & Shear.

Sepedonium chrysospermum Fr.

Young fruit-bodies of *Melanogaster variegatus* Vitt. var. *broomeianus* Berk., collected in September 1953, at Blaise Castle, Bristol (no. 871 in my collection), were covered with a bright orange fungal mycelium, which extended into the surrounding soil for a distance of at least 5 cm. Older fruit-bodies were soft and rotting. The colour was due to masses of orange, echinulate chlamydospores of *Sepedonium chrysospermum*. The fungus was easily isolated from the interiors of rotting fruit-bodies and from the surrounding soil.

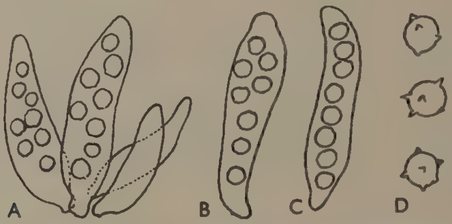

Fig. 2. *Battarina inclusa*. A, group of immature asci from squash preparation of perithecium; B–C, nearly mature asci showing irregular and uniseriate arrangement of asci respectively. A–C, × 800. D, mature ascospores, showing scattered spines, × 1000.

This fungus was also found on *Rhizopogon provincialis* Tul. at Knightwood, in the New Forest, September 1954. No morphological differences between this and the form on *Melanogaster* could be detected.

Tulasne (1860) reported this species on *Melanogaster variegatus* and on *Agaricus* [*Paxillus*] *involutus*, *Boletus* spp., *Scleroderma verrucosa* and *Octaviania* [*Arcangeliella*] *asterosperma*. In the Society's 'List of Hyphomycetes recorded for Britain' (Wakefield & Bisby, 1941) *Sepedonium chrysospermum* is recorded only on Hymenomycetes, chiefly species of *Boletus*. The perithecial stage, *Apiocrea chrysosperma* (Tul.) Syd. is recorded for Britain by Petch (1938) only on boleti, *Paxillus involutus* and *Scleroderma* sp.

Neither the perithecial stage (said by Petch (1938) to be rare) nor the conidial stage (*Sporotrichum chrysospermum* Harz, sometimes associated with it (Wakefield & Bisby, 1941)) could be detected on the *Melanogaster* material.

Heim (1948) considers that the fact that *Hypomyces* [*Apiocrea*] *chrysospermum* parasitizes certain species of *Rhizopogon* as well as *Boletus* spp. and *Paxillus involutus* is a 'biological argument in favour of a relationship between the three genera'. It might equally be used as evidence for a relationship between *Melanogaster*, *Boletus* and *Paxillus*. All three genera have smooth-walled yellow to brown basidiospores. The development of the fruit-body and the basidial characters of *Rhizopogon* and *Melanogaster* suggest a relationship between these two genera (Hawker, 1954; and in

Press). The same parasite has, however, also been recorded on *Scleroderma verrucosa* and *Arcangeliella asterosperma*, the spores of which are spherical and sculptured or echinulate. Moreover, the development of the fruit-bodies of *Arcangeliella*, so far as it is known, is not closely similar to that of fruit-bodies of *Rhizopogon* and *Melanogaster*, and Knapp (1954) separates the last two genera, placing *Rhizopogon* with *Hymenogaster*. It is doubtful, therefore, whether Heim was justified in considering the possession of a common parasite as a 'biological argument' in favour of a particular relationship.

The writer wishes to thank Dr R. W. G. Dennis of the Herbarium, Royal Botanic Gardens, Kew, for access to material of species of *Rhizopogon* and to type material of *Battarina inclusa*, to Mr N. Y. Sandwith for the Latin diagnosis of *Rhizopogon reticulatus*, and to Mr T. J. Wallace and Dr J. Manners for material of *Rhizopogon* spp.

REFERENCES

BERKELEY, M. J. & BROOME, C. E. (1861). Notices of British Fungi. *Ann. Mag. Nat. Hist.*, Ser. 3, **7**, 451–452.

BISBY, G. R. & MASON, E. W. (1940). List of Pyrenomycetes recorded for Britain. *Trans. Brit. mycol. Soc.* **24**, 127–243.

HAWKER, L. E. (1954). British Hypogeous Fungi. *Phil. Trans.* B, **237**, 429–546.

HAWKER, L. E. (in the Press). Hypogeous Fungi. *Biol. Rev.*

HEIM, R. (1948). Phylogeny and natural classification of macrofungi. *Trans. Brit. mycol. Soc.* **30**, 161–178.

KNAPP, A. (1954). Die europäischen Hypogaeen-Gattungen und ihre Gattungstypen. II. Teil. *Schweiz. Z. Pilzk.* **32**, 33–44.

PETCH, T. (1938). British Hypocreales. *Trans. Brit. mycol. Soc.* **21**, 243–305.

TULASNE, L. R. (1860). De quelques Sphéries fongicoles. *Ann. Sci. nat.*, Sér. 4, **13**, 16.

WAKEFIELD, E. M. & BISBY, G. R. (1941). List of Hyphomycetes recorded for Britain. *Trans. Brit. mycol. Soc.* **25**, 53–126.

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THE EFFECT OF TRACE METAL DEFICIENCY ON SOME *TRICHOPHYTON* STRAINS

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(With Plate 2)

An attempt was made to elucidate the trace metal requirements of two strains of *Trichophyton mentagrophytes* and two of *T. rubrum*. Adsorption of the metals with $CaCO_3$ was found to be the most efficient of the recognized methods for the removal of these elements from a medium containing a complex nitrogen source. No species difference in the Fe, Zn, Mn and Cu requirements of the fungi was indicated by the effect of deficiency of these metals on the weight of the mycelial mat, but three of the strains were shown to be very sensitive to Fe deficiency, three to Zn deficiency and one *T. rubrum* strain to Mn deficiency as well. The red pigment of this strain became bright yellow in Mn deficient medium. No very marked changes in morphology were noted.

INTRODUCTION

Very little work has been done on the trace metal requirements of the dermatophyte fungi, probably owing to the difficulties raised by the obligate requirement of most of them for a complex source of nitrogen if they are to attain maximum development. Only Mosher, Saunders, Kingery & Williams (1936) reported certain results, but gave no details of their methods, and Stockdale (1953) studied the requirements of *Trichophyton persicolor*, but found difficulty in obtaining consistent results. Very successful methods for removing trace metals from media containing inorganic sources of nitrogen have been developed by Steinberg (1950), Nicholas & Fielding (1951) and Donald, Passey & Swaby (1952), but the latter workers were much less successful when they applied their methods to broth and peptone media.

REMOVAL OF TRACE METALS

Of the two groups of methods available, Donald *et al.* and Steinberg have found adsorption to be superior to chelation, at least in the removal of Fe, Zn, Mn and Cu, both in the results achieved and in the simplicity of the technique, and the former workers, contrary to the opinion of Stockdale, preferred Al_2O_3 to $CaCO_3$ as an adsorbent. In preliminary experiments with casein-sucrose and casein-glucose media $CaCO_3$ gave us considerably better results than Al_2O_3, and we also noted slightly better removal from the sucrose than from the glucose medium. This observation could be explained by the presence of complex compounds in the glucose medium, formed when the amino-acids and aldehydic sugar are heated together

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(Frankel & Katchalsky, 1937), compounds which might well adsorb and hold the trace metals. However, as the mycelial weights of our *Trichophyton* strains were reduced to about a quarter of the maximum when sucrose was used as the carbon source, the casein-glucose medium treated with $CaCO_3$ by Steinberg's method was finally adopted as the routine method.

MATERIALS AND METHODS

Fungi. Four isolates of *Trichophyton* were tested: two, *T. mentagrophytes* 1 (Ti 1) and *T. rubrum* 2 (Tr 2) were given to me by Dr Jacqueline Walker, who had isolated them from cases of tinea about 6 months previously; *T. rubrum* 5 (Tr 5) was isolated in this laboratory from a long-standing case of tinea unguium, shortly before the work began; and *T. mentagrophytes* 4 (Ti 4) had been obtained some years previously from a small outbreak of ringworm in guinea-pigs and man. None of the isolates showed any signs of pleomorphism at the beginning of the experiments. The test fungus used to determine the amount of trace metals present in the media was a strain of *Aspergillus niger* originally isolated by Gerretsen and kindly given to one of us (M.P.E.) by Dr Mary Gregg.

Media. These were made up with 'Analar' grade chemicals, not recrystallized before use, and the N source was 'Difco' Casamino Acids. All water used was triple-distilled from glass and contained 0·016 p.p.m. of trace metals (kindly determined for us by Mr P. Morries of the British Drug Houses Ltd.). The medium was an adaptation of that of Burkholder & Moyer (1943) containing: dextrose (or sucrose) 50 g.; casein, 5 g.; KH_2PO_4, 1·5 g.; $MgSO_4 . 7H_2O$, 0·5 g.; $CaCl_2 . 2H_2O$, 0·33 g.; distilled water, 1 l. Preliminary work using only 20 g. glucose and 2 g. casein showed that while there was insufficient nutrient to support maximum growth of the fungi, it was no easier to eliminate trace metals from the weaker medium. The amounts of trace metals added to complete the medium for the dermatophytes were varied to suit experimental requirements: for *Aspergillur niger* the quantities recommended by Nicholas & Fielding (1951) were used.

Preparation of glassware. Adequate cleaning of glassware used at every stage of these experiments was essential for success, and the minimum treatment necessary was as follows: soak for 24 hr. in acid dichromate made with 98 % H_2SO_4, rinse 6 times in tap water and 3 times in triple-distilled water, drain and cover immediately with parchment paper. Glassware was cleaned immediately before each experiment, as it was found that with treated medium cleaned flasks stored for 3 weeks produced double the weight of mycelium produced in freshly cleaned flasks. It is uncertain whether this was due to contamination from the atmosphere, or leaching of metals from the glass by any moisture remaining in the flasks. Flasks were plugged with gauze-covered cotton-wool plugs, as the substitution of glass covers produced no better results.

Culture methods. Stock cultures were kept on malt agar (*A. niger*) and Sabouraud's glucose agar (the dermatophytes); no precautions were taken to remove trace metals from these cultures as Stockdale (1953) had found no advantage in so doing. Freeze-dried cultures of all the fungi were available. Inoculum for experiments consisted of spores rubbed off a slope culture and suspended in triple-distilled water. One drop of this suspension was added from an acid-cleaned pipette to each 250 ml. conical flask containing 50 ml. of medium. *A. niger* cultures were harvested after 1 week's incubation at 30° C., the dermatophytes after 3 weeks', all cultures being killed by autoclaving, filtered from the medium, dried overnight at 98° C., and weighed. Each treatment was carried out in duplicate.

RESULTS

By growing *A. niger* on treated medium to which adequate amounts of all but one of the trace metals had been returned it was possible, by comparing the weight and appearance of the mycelial mats so obtained with the graphs and illustrations given by Nicholas (1952) and Nicholas & Fielding (1951), to gauge approximately how much of each trace metal remained after treatment. The figures, per 50 ml. of medium, are as follows, the optimum amount for *A. niger* being given in brackets: Fe, 0·8 μg. (20); Zn, 1·1 μg. (20); Cu, 0·8 μg. (2·5); Mn, 0·01 μg. (5). Though the actual removal of Cu is of the same order as that of Fe and Zn, the total amount necessary for growth is so small that relatively little difference could be shown between the depleted and the control medium for this element.

Optimum concentration of metals. Approximate figures were obtained for the dermatophytes by adding varying amounts of the trace metals to treated medium until maximum mycelial weights were obtained, the total amount of each metal present being reckoned as that added, together with that calculated as remaining in the medium after treatment. The figures were as follows: Cu, 1·3 μg. (1·8 μg. for Ti 1 and Tr 5); Fe, 5·8 μg.; Zn, 8·1 μg; Mn, 1·01 μg per 50 ml. medium. The concentrations of the metals found by Nicholas to be optimal for *A. niger* are somewhat higher than those for the dermatophytes, but a possible explanation for this discrepancy lies in the fact that a mature mat of *A. niger* weighs from 800 to 900 mg., and so might be expected to use more nutrient than a dermatophyte weighing from 400 to 500 mg.

Effect of deficiency of metals. This was measured by the reduction in weight of the mycelial mat, and microscopic and macroscopic changes in the fungi were also recorded. The results of two experiments using isolates which had been freeze-dried appear in Table 1, which also shows the degree of reproducibility obtained throughout this investigation. Pl. 2 illustrates the macroscopic differences obtained in two of the isolates examined.

The results do not indicate a species difference in the metal requirements of these fungi, but they do show clearly that three of them at least are much more sensitive to Fe deficiency than is *A. niger*, which can produce about 250 mg. of mycelium under conditions in which the dermatophytes produce about 40 mg. The effect of lack of Fe on isolate Ti 1 is the only occasion in these experiments where inconsistent behaviour was observed, for in all experiments previous to those whose results are tabled the mycelial weight in Fe-deficient medium had been similar to that for the other three fungi; only in the final two experiments when freeze-dried cultures were used, did it rise appreciably. Isolate Ti 4 seems to need considerably less Zn than did the others.

Tr 5 is interesting, in that as well as showing a very marked loss of weight in both Fe- and Zn-deficient media, it is the only isolate to react strongly to lack of Mn, Ti 1 being apparently unaffected and the other two only slightly inhibited under the same conditions. Tr 5 was also the only isolate to become strongly pleomorphic during these experiments and this necessitated the use of freeze-dried cultures during the latter part of the work. In accordance with the observations of Robbins & Ma (1945) the mycelial weight of the pleomorphic form (600 mg.) was considerably greater than that of the normal.

Table 1. *Mycelial weight (mg.) of the dermatophytes grown in metal-deficient media*

Trichophyton mentagrophytes

	Ti 1		Ti 4	
Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2
All metals	490·4	510·9	471·9	505·5
No metals	40·6	59·1	36·9	52·4
No Cu	358·1	483·2	445·5	515·5
No Mn	475·5	497·3	393·0	427·3
No Fe	125·7	223·7	35·4	63·8
No Zn	76·6	65·5	191·8	246·7

Trichophyton rubrum

	Tr 2		Tr 5	
Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2
All metals	458·1	508·3	394·7	352·9
No metals	29·1	30·5	28·6	26·5
No Cu	452·0	467·8	294·5	299·1
No Mn	319·6	359·7	160·3	139·6
No Fe	45·1	35·2	38·9	37·1
No Zn	88·9	184·4	58·2	74·3

Only one change in pigmentation was noted, and this also occurred in isolate Tr 5, where Mn deficiency was correlated with the change of the usual deep crimson-brown pigment to a bright golden yellow.

The pH of the medium at the end of the growth period was determined in the two experiments whose results are given, and shows a general trend towards acid production and lowering of the pH by about 1: this, however, was much less marked in isolate Ti 1 than in the other three. One exception was the effect of Zn deficiency on the two *T. mentagrophytes* strains, when the pH dropped by about 1 below the usual figures though the amount of mycelium produced was comparatively small. The only record of a rise in pH was for Ti 1 in Fe deficiency, when a pH of 8 was reached, and it seems possible that there may be some relationship between this and the unusual weight of mycelium produced by this fungus, as already mentioned, in the final experiments of the series.

Finally, certain observations were made on the character of the mycelium and the sporulation of the fungi. Where the mycelial mat weighed less than 200 mg. it was almost entirely submerged, mucoid, and lacking in spores though sometimes, particularly in Fe-deficient media, there was some growth and sporulation round the sides of the flasks (Pl. 2). All four isolates formed microspores in the complete medium, Ti 1 in vast quantities; and hyphal swellings, sometimes of great size, were also universal. Spirals occurred in Ti 1 only but racquet hyphae were of varying abundance in all the fungi. It proved impossible to correlate any of these structures with sufficiency or deficiency of any one trace metal, the general rule being that the poorer the growth the fewer the specialized organs. It was noteworthy, however, that the Mn deficiency which, at the level attained in this work, almost completely prevented sporulation in *A. niger*, apparently had no effect on microspore formation in the dermatophytes. Macrospores were produced only by isolate Ti 1, and then not invariably: when present they varied in number from a very few to comparative abundance and in nearly every case could be correlated with poor growth, most often appearing on the sides of the flask of an Fe-deficient culture.

Our thanks are due to Dr G. C. Ainsworth, at whose suggestion this work was undertaken; to Mrs L. Baker, Miss F. Crewe and Miss D. Hart for technical assistance; to Mr A. T. Mitchell and Mr D. V. Millington for taking and retouching the photographs; to Dr S. W. F. Underhill for his interest and encouragement, and to the Directors of The British Drug Houses Ltd. for permission to publish this account.

REFERENCES

BURKHOLDER, P. R. & MOYER, D. (1943). Vitamin deficiencies of fifty yeasts and moulds. *Bull. Torrey bot. Cl.* **70**, 372–377.

DONALD, C., PASSEY, B. I. & SWABY, R. J. (1952). A comparison of methods for removing trace metals from microbiological media. *J. gen. Microbiol.* **7**, 211–220.

FRANKEL, M. & KATCHALSKY, A. (1937). The interaction of α-amino-acids and peptides with sugars in aqueous solution. *Biochem. J.* **31**, 1595–1604.

MOSHER, W. A., SAUNDERS, D. H., KINGERY, L. B. & WILLIAMS, R. J. (1936). Nutritional requirements of the pathogenic mould *Trichophyton interdigitale*. *Plant Physiol.* **11**, 795–806.

NICHOLAS, D. J. D. (1952). The use of fungi for determining trace metals in biological materials. *Analyst*, **77**, 629–642.

NICHOLAS, D. J. D. & FIELDING, A. H. (1951). The use of *Aspergillus niger* (M) for the determination of magnesium, zinc, copper and molybdenum available in soils to crop plants. *J. hort. Sci.* **26**, 125–147.

ROBBINS, W. J. & MA, R. (1945). Growth factors for *Trichophyton mentagrophytes*. *Amer. J. Bot.* **32**, 509–523.

STEINBERG, R. A. (1950). Trace element impurities in nutrient solutions for fungi. *Arch. Biochem.* **28**, 111–116.

STOCKDALE, P. M. (1953). Requirements for the growth and sporulation of *Trichophyton persicolor*. *J. gen. Microbiol.* **8**, 434–441.

EXPLANATION OF PLATE 2

The effect of trace metal deficiency on *Trichophyton* spp.

Fig. 1. *T. mentagrophytes* 4.

Fig. 2. *T. rubrum* 5.

a, Zn deficiency; *b*, complete medium; *c*, Zn, Fe, Mn and Cu deficiency; *d*, Mn deficiency; *e*, Fe deficiency; *f*, Cu deficiency.

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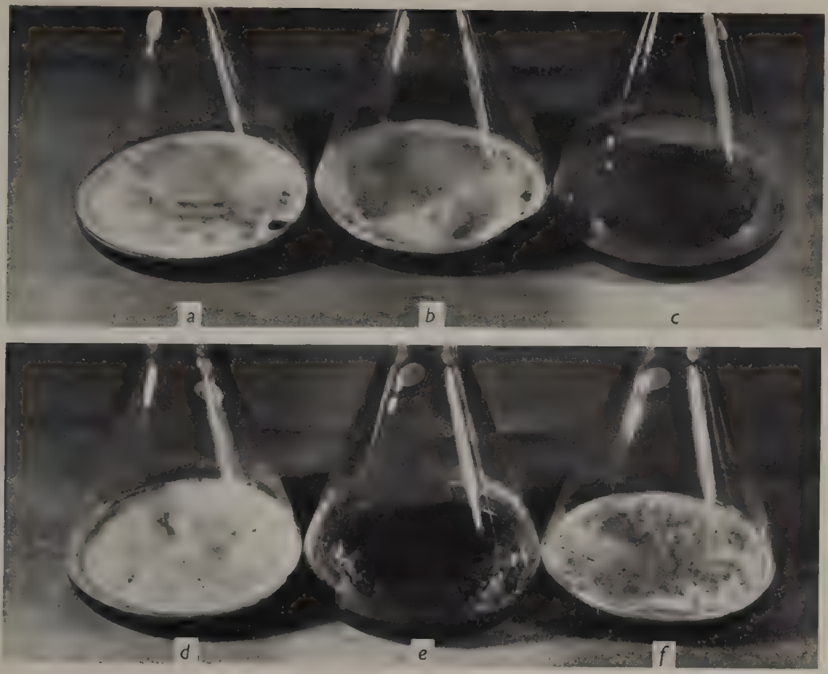

Fig. 1.

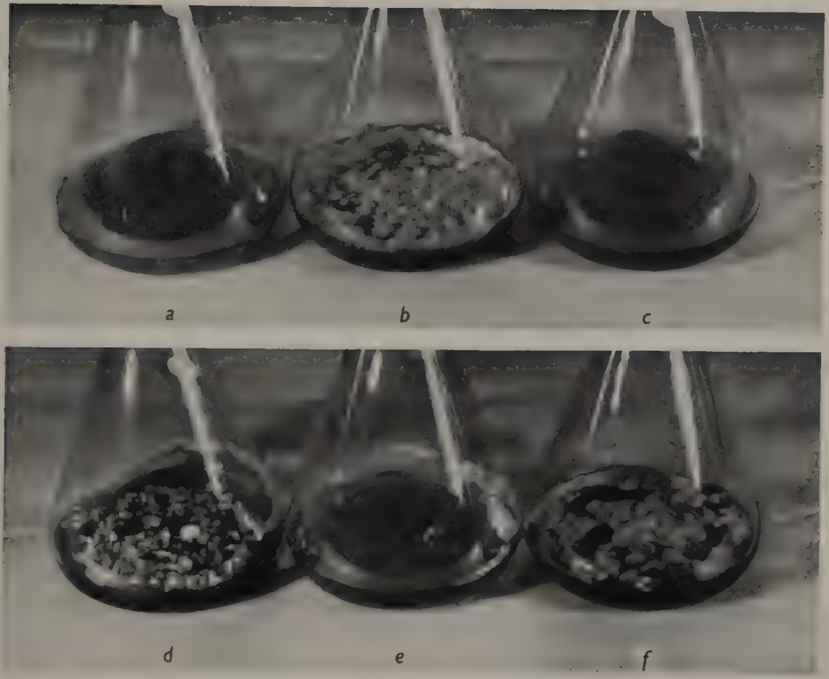

Fig. 2.

Trans. Brit. mycol. Soc. **38** (1), 83–87 (1955).

A SELECTIVE MEDIUM FOR THE ENRICHMENT CULTURE OF *PSEUDOMONAS MORS-PRUNORUM* WORMALD

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A selective medium for the enrichment culture of *P. mors-prunorum*, contains sodium tartrate and the anionic surface active agent di-octyl sodium sulphosuccinate.

Introduction

Although specialized media have long been employed for the separation and identification of bacteria pathogenic to animals, their use for the isolation of phytopathogenic bacteria has been exploited for few species, e.g. *Corynebacterium sepedonicum* (Burkholder, 1938; Snieszko & Bonde, 1943), *Bacterium tumefaciens* (Patel, 1926), *Xanthomonas stewarti* (Ivanoff, 1933) and *Pseudomonas phaseolicola* (Wilson, 1938).

The medium for the enrichment culture of *P. mors-prunorum*, the cause of the Bacterial Canker and Leaf Spot disease of stone fruits (Wormald, 1932) was originally developed to study the possible occurrence of small populations of this stone fruit pathogen overwintering epiphytically on dormant buds and other tree surfaces. It has since proved extremely valuable for making isolations from diseased bark and leaf tissues in summer and autumn, respectively, when very few viable cells of *P. mors-prunorum* are normally encountered in lesions.

Preparation of the medium

The medium, which contained sodium tartrate as the carbon source and the anionic surface active agent di-octyl sodium sulphosuccinate, had the following composition:

Sodium tartrate	10·0 g.	$MgSO_4 . 7H_2O$	0·5 g.
Di-octyl sodium sulphosuccinate	0·1 g.	KCl	0·5 g.
$NaNO_3$	2·0 g.	$FeSO_4 . 5H_2O$	Trace
K_2HPO_4	1·0 g.	Glass distilled water	1000 ml.

The $MgSO_4 . 7H_2O$, KCl and $NaNO_3$ were dissolved in 500 ml. of distilled water, autoclaved at 15 lb. for 15 min. and cooled. The K_2HPO_4, sodium tartrate and di-octyl sodium sulphosuccinate were dissolved separately in 150, 100 and 250 ml. of distilled water, respectively, and added without sterilization to the first solution. A few crystals of the iron salt were added and the medium adjusted with HCl to approximately pH 5·5. The broth was distributed in tubes (previously autoclaved empty at 15 lb. for

15 min.) and sterilized by bringing the pressure slowly (3–5 min.) up to 7 lb. and then turning off the autoclave.

The incorporation of 0·1 % di-octyl sodium sulphosuccinate in the medium resulted in a turbidity after autoclaving which tended to mask growth, although the growth of *P. mors-prunorum* was not inhibited. Turbidity was avoided by using 0·01 % without any apparent loss in the selectivity of the medium.

GROWTH OF *PSEUDOMONAS MORS-PRUNORUM* IN THE MEDIUM

Growth was blue-opalescent without any pellicle, and became evident at 25° C. after 18 hr. with large inocula, and after 4–7 days with small inocula.

Specificity. Erikson (1945) reported that some strains of the organism did not readily utilize tartrate. To test this further, thirty strains, isolated directly from diseased plum and cherry tissues on nutrient agar + 5 % sucrose, were sown in the medium. Some variation in growth was observed, but no evidence was found that the medium was markedly specific for certain strains.

Effect of pH *on the medium.* The effect of the initial pH of the medium on the growth of two strains, C 12 R and C 13, both from cherry, was investigated. Both strains grew over the entire range tested, i.e. pH 5·0–7·2 with maximum growth at approximately pH 6·5. When the selectivity of the medium (see below) at different pH values was compared, best results were obtained with an initial pH of 5·5. Fewer contaminant species were encountered, possibly because of the increased bactericidal activity of the di-octyl sodium sulphosuccinate at the lower pH (Gershenfeld & Perlstein, 1941). Growth resulted in an increase in pH at all levels.

Size of inoculum. A suspension of *P. mors-prunorum* (strain C 12) was prepared from 48 hr. growth on nutrient agar + 2 % glycerine and diluted serially by one-tenths to extinction. Transfers were made from the lower dilutions to tubes of tartrate broth using a 4 mm. loop, or a micropipette. The approximate number of viable cells in the various inocula was estimated by plate counts on nutrient agar + 5 % sucrose. After 8–10 days at 25° C. visible growth was detected in tubes sown with approximately ten cells; with heavier sowings of approximately 200 cells, a distinct turbidity was evident after 5–6 days. These results were taken to indicate that, subject to possible variation from strain to strain, *P. mors-prunorum* would grow in the tartrate broth from comparatively small inocula.

SELECTIVITY OF MEDIUM

The selectivity of the tartrate broth was compared with that of: (*a*) a sucrose broth with the same inorganic constituents as the tartrate broth but with 5 % sucrose as the carbon source; and (*b*) nutrient broth (Difco) + 5 % sucrose. Neither (*a*) nor (*b*) contained di-octyl sodium sulphosuccinate and in both *P. mors-prunorum* grew vigorously. Selectivity was determined by test isolations from necrotic leaf spots dissected from infected plum and cherry leaves in the autumn.

In spring the pathogen is present in large numbers in these leaf spots from which it can be readily isolated in pure culture by macerating spots in a drop of sterile water and streaking the resultant suspension directly on to agar plates. As the summer progresses the pathogen tends to die out and the number of associated saprophytes tends to increase. By autumn the number of viable cells in individual spots is very small and isolation by conventional methods extremely difficult.

Necrotic spots were macerated in sterile water on sterile cover-slips and transferred to tubes of appropriate broth at the rate of one spot per tube. The tubes were incubated at 25° C. and examined periodically over the following 2 weeks and any growth plated on to nutrient agar + 5 % sucrose for preliminary identification. On this medium the growth rate and colony structure of *P. mors-prunorum* are together characteristic (Wormald, 1932). Identity was checked by growth characteristics in nutrient broth + 5 % sucrose and on 2 % purple lactose agar (Wormald, 1932) and in gelatin and lactose and maltose broths (Crosse, 1953). Confirmation of identity was obtained by prick inoculation of randomly selected isolates from different leaf spots into cherry buds.

The results of representative tests carried out in the autumn of three successive years are shown in Table 1.

In all three tests the tartrate broth proved markedly superior for the enrichment of *P. mors-prunorum* under the conditions employed, particularly in 1952 when the comparison was made with a non-selective nutrient broth + 5 % sucrose. It will be noted that in 1951, when concomitant saprophytic species were apparently less abundant, several isolates were obtained in the sucrose broth. This medium may also be regarded, to some extent, as selective since it will not support the growth of organisms other than those, like *P. mors-prunorum*, with simple growth requirements. An outstanding result was obtained in 1952 with the tartrate broth adjusted to pH 5·5. Of the ten tubes showing growth nine yielded *P. mors-prunorum*, seven of which were pure cultures.

Table 1. *Comparison of tartrate and sucrose medium*

				No. of spots yielding			
Year	Medium	pH	No. of spots tested	No growth	*P. mors-prunorum* (total)	*P. mors-prunorum* (pure culture)	Fungal growth
1950	Tartrate broth	7·0	35	2	15	5	0
	Sucrose broth	7·0	35	0	0	0	4
1951	Tartrate broth	7·0	50	16	32	11	0
	Sucrose broth	7·0	50	0	23	2	5
1952	Tartrate broth	5·5	20	10	9	7	0
	Nutrient broth + sucrose	7·0	20	0	0	0	7

A feature of the tartrate broth was the number of tubes remaining free of growth. Fungal growth was never recorded in this medium. In a few tubes periods of 10 days elapsed before growth was detected. These invariably yielded pure cultures of *P. mors-prunorum* and presumably indicated growth from comparatively small inocula.

The following examples also serve to illustrate the selectivity of the medium:

In December 1951 the surfaces of cherry buds on trees of the variety Schrecken were smeared with bacterial slime of *P. mors-prunorum* and left exposed to normal winter weather conditions until April of the following year when the buds were removed from the trees and transferred to tubes of tartrate broth. Of 200 buds treated in this way, nine yielded pure cultures of the pathogen. No other organism was encountered and the remaining tubes remained free of growth.

Pathogenic isolates of *P. mors-prunorum* have been readily obtained from the surface of 2 mm. disks of leaf tissue in the broth and in one instance a virulent strain was obtained in this way in December from a piece of rotted leaf.

Discussion

Although the medium is not entirely specific to *P. mors-prunorum*, with the exception of an unidentified species of *Pseudomonas*, none of the saprophytic bacteria that were encountered from dead cherry and plum tissues grew well in it. The selectivity achieved is attributed mainly to two factors, namely the use of a carbon source less generally available to other bacteria and to the incorporation of the surface active agent as a bactericide.

The choice of sodium tartrate was based on the known ability of certain phytopathogenic *Pseudomonas* spp. to dissimilate the salts of organic acids (Wilson, 1936; Erikson, 1945) and was made after a comparison with other organic salts, e.g. sodium malate, succinate, fumarate, malonate and citrate. In its ability to dissimilate sodium tartrate *P. mors-prunorum* appears to belong to a minority group of the plant pathogenic *Pseudomonas* spp. (Burkholder & Starr, 1948).

There is no need to stress the extensive use already made of colloidal electrolytes, particularly of the bile salts and 'basic' dye stuffs, in the preparation of differential media. It is, however, interesting to note that di-octyl sodium sulphosuccinate proved markedly superior to the bile salt sodium tauroglycocholate in suppressing saprophytes in the present medium. It also had the added advantage of being partially fungistatic, a property of some importance when isolating from dead plant tissues.

The insensitivity of *P. mors-prunorum* to di-octyl sulphosuccinate suggests that this and similar compounds may prove useful for the isolation of other phytopathogenic *Pseudomonas* spp. It is perhaps worth mentioning in this connexion that the use of certain 'basic' dyes, e.g. brilliant green and crystal violet, in the same medium as the anionic detergents should be approached with caution. Although mixtures of anionic and cationic colloidal electrolytes, e.g. sodium taurocholate and crystal violet (Patel, 1926) have been successfully used in selective media, it should be noted that there is a strong tendency for mutual precipitation between these substances, which may considerably reduce the ability of the mixture to inhibit bacterial growth (Alexander & Tomlinson, 1949).

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REFERENCES

ALEXANDER, A. E. & TOMLINSON, A. J. H. (1949). Colloidal electrolytes, their biological activity and influence upon other biologically active compounds, with particular reference to soaps and soap/phenol mixtures. IV. Some simple experiments to illustrate the effect upon bacterial growth of mutual precipitation in mixtures of colloidal electrolytes. *Surface Chemistry, a report of symposium on recent advances in Surface Chemistry*, Bordeaux, 1947, p. 325. Butterworth Scientific Publications, London.

BURKHOLDER, W. H. (1938). The occurrence in the United States of the tuber ring rot and wilt of potato (*Phytomonas sepedonica* (Spieckermann u. Kotthoff) Bergey *et al.*). *Amer. Potato J.* **15**, 243–245.

BURKHOLDER, W. H. & STARR, M. P. (1948). The generic and specific characters of phytopathogenic species of *Pseudomonas* and *Xanthomonas*. *Phytopathology*, **38**, 494–502.

CROSSE, J. E. (1953). Bacterial diseases of stone-fruit trees in Britain. IX. Bacteriosis of Apricot. *Trans. Brit. mycol. Soc.* **36**, 38–45.

ERIKSON, D. (1945). Certain aspects of resistance of plum trees to bacterial canker. I. Some biochemical characteristics of *Pseudomonas mors-prunorum* (Wormald) and related phytopathogenic bacteria. *Ann. appl. Biol.* **32**, 44–52.

GERSHENFELD, L. & PERLSTEIN, D. (1941). Significance of hydrogen-ion concentration in the evaluation of the bactericidal efficiency of surface tension depressants. *Amer. J. Pharm.* **113**, 89–92.

IVANOFF, S. S. (1933). Stewart's wilt disease of corn, with emphasis on the life history of *Phytomonas stewarti* in relation to pathogenesis. *J. agric. Res.* **47**, 749–770.

PATEL, M. K. (1926). An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. *Phytopathology*, **16**, 577.

SNIESZKO, S. F. & BONDE, R. (1943). Studies on the morphology, physiology, serology, longevity and pathogenicity of *Corynebacterium sepedonicum*. *Phytopathology*, **33**, 1032–1044.

WILSON, E. E. (1936). Symptomatic and etiologic relations of the canker and blossom blast of *Pyrus* and bacterial canker of *Prunus*. *Hilgardia*, **10**, 213–240.

WILSON, R. D. (1938). A selective medium for *Bacterium medicaginis* var. *phaseolicola* (Burkholder) Link & Hull. *J. Aust. Inst. agric. Sci.* **4**, 47–49.

WORMALD, H. (1932). Bacterial diseases of stone fruit trees in Britain. IV. The organism causing Bacterial Canker of plum trees. *Trans. Brit. mycol. Soc.* **17**, 157–169.

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A BACTERIAL DISEASE OF SHIRLEY POPPIES IN TANGANYIKA

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(With Plate 3)

The symptoms of a bacterial disease of Shirley poppy (*Papaver rhoeas*) are described and an account is given of the morphological, cultural and biochemical characters of a species of *Pseudomonas* which was shown to cause the disease. It is considered that this Pseudomonad has not been previously described and the name *Pseudomonas papaveris* sp.nov. is proposed for it.

Occurrence and symptoms

A disease of Shirley poppies (*Papaver rhoeas* L.) was first seen in a garden near Moshi, Tanganyika, in July 1944 (Wallace, 1944). Since that time it has appeared each year with varying severity, on self-sown poppy plants growing in the same beds. This garden is the only place where Shirley poppies have been seen in Tanganyika. The disease appears in July, August and September, which is the normal growing season for these plants. In this district there is a period of heavy rainfall during April and May and from July to September the average precipitation diminishes from 2½ in. in July to 1 in. in September.

Sometimes the first symptom is the appearance of a purplish black colour on the stems at or near ground-level. This develops into a black streak spreading upwards to the branch tips (Pl. 3, fig. 1). The discoloration may involve the whole stem or, initially, one side only. Spread is more rapid up the stem than across. The foliage wilts, turns first yellow and then brown, with the infected veins appearing as black lines. Finally complete disintegration of the blackened parts occurs, resulting in a foul-smelling slimy mass. At other times black spots appear in the leaf veins or blades, or in lateral branches well above soil level. Such infections may spread and kill the plants or they may remain localized without having a fatal effect. If a blackened stem is cut across, an exudation of bacterial slime is often seen. Plants of all ages are attacked, and well-grown plants may be killed outright in a matter of days or a week or two.

Pathogenicity experiments

In preliminary infection experiments in 1944, diseased tissue was used as inoculum and pricked into healthy stems; a typical dark discoloration was produced after 24 hr. Where infective material was placed on unwounded tissue no infection was observed.

In 1950 bacteria were isolated from naturally diseased tissue and grown in pure culture. A suspension of the bacteria in sterile water was sprayed on to young Shirley poppy plants and typical leaf-spotting was observed after 48 hr. Needle puncture inoculations into leaf veins similarly produced positive results after the same time, while after 4 days the lesions were up to 11½ cm. long and infection had penetrated into the leaf blades (Pl. 1, fig. 2). In some cases the infection spread to the crowns of the plants and the plants were killed; in other cases the lesions remained more localized, and the plants survived, although the infected leaves were killed. These tests were repeated with exactly similar results. The causal bacterium was re-isolated from the artificially inoculated plants.

Lettuce seedlings and young leaves of *Hibiscus rosa-sinensis* plants could not be infected by spraying or by needle-prick inoculations. Citrus fruits (sweet and Seville orange, grapefruit and lemon) and slices of potato, carrot and onion were not affected when inoculated through needle-pricks. Young Oriental poppy plants (*Papaver orientale* L.) when inoculated showed symptoms similar to those on Shirley poppy.

IDENTIFICATION OF CAUSAL ORGANISM

Single colonies were plated out several times to ensure purity and then tested for pathogenicity on Shirley poppies. Re-isolates from poppies were also studied and were not found to differ culturally or biochemically from the bacterium originally isolated. Except where stated the incubation temperature was 25° C. and the methods used to characterize the organism were those recommended in the *Manual of Methods for Pure Culture Study of Bacteria* (S.A.B. 1953). All the tests were replicated at least 3 times for each culture. Carbohydrate utilization tests were run for 28 days.

Morphology

The bacterium is rod-shaped with rounded ends and occurs singly or in pairs. It is motile with one to several polar flagella and is Gram-negative. Cells from a 48 hr. nutrient agar culture, measured in a hanging drop, vary in size from 0·8–4μ in length and 0·35–0·6μ in width (mean, about 2 by 0·4μ).

Cultural characters

Colonies on beef extract-peptone agar plates are at first greyish white, translucent, circular, smooth, entire and raised, but after 8 days they become olive-buff with slightly undulate margins, denser centres and show a tendency to an umbonate elevation. On potato-dextrose agar plates, colonies are whitish, translucent, circular, smooth, undulate and at first raised, but after 8 days umbonate with denser centres. Colonies on both media reach about 2 mm. diameter in 8 days and have an internal flocculence which at first tends to a concentric arrangement but later becomes more confused, and which finally disappears. On beef-extract slopes, growth is moderate, filiform, glistening but becoming dull with age, and slightly viscid; there is good production of a yellowish green fluorescent water-soluble pigment but no browning of the medium. Nutrient broth is slightly clouded in 2 days and a very thin, easily broken pellicle and a viscid precipitate form after 4 days. Litmus milk becomes alkaline, and after 12 days the litmus in the bottom centimetre of the tube is reduced; no further reduction occurs when the tubes are incubated for 6 weeks. Growth on sterile potato plugs is thin and at first white, later becoming brownish. The pathogen will not grow in 6 % sodium chloride in tryptone-yeast broth but grows very slightly in 5 % and moderately in 4 %. In shake cultures growth is restricted to the upper millimetre of the agar. The optimum temperature for growth is 25–30° C. and the maximum 35° C. The thermal death-point as determined by Magoon's method (1926) is 49–50° C. for an exposure time of 10 min.

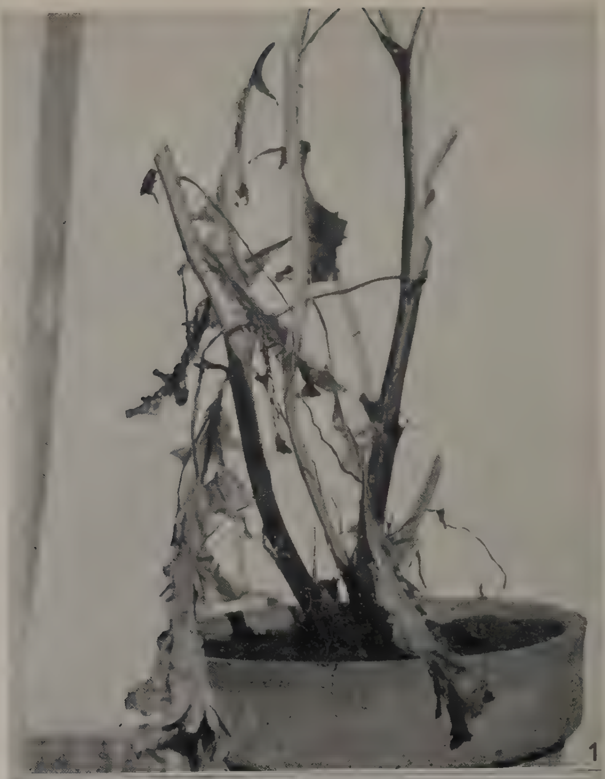
Biochemical characters

Nutrient gelatin stabs are not liquefied even after 8 weeks at room temperature. Good growth is made on tryptone broth, but neither indole nor hydrogen sulphide can be detected on this medium using oxalic acid or lead acetate papers, respectively. No production of nitrite from nitrate can be detected using normal test conditions. Starch is partially hydrolysed to an erythrodextrin-like substance which gives a red-brown colour with iodine; no lypolytic activity can be observed in 14 days, using Cabral's modification of Starr's method (Dowson, 1949).

Xylose, dextrose, mannose, glycerol, maltose, lactose and sorbitol as 1 % solutions in synthetic medium (Ayres, Rupp & Johnson, 1919) can serve as sole sources of carbon, but rhamnose, sucrose, raffinose and salicin cannot. After 28 days' growth in xylose, dextrose, mannose or glycerol, the medium is acid to bromcresol purple, while in maltose and lactose the medium is acid to bromthymol blue but not bromcresol purple. There is no acid production from sorbitol in 28 days.

Taxonomy

The characters of the poppy pathogen obviously place it among the 'green fluorescent' species of the genus *Pseudomonas* Migula and so distinguish the disease from the two previously recorded diseases of poppy caused by *Bacillus papaveris* Ayyar (probably a synonym of *Erwinia aroidae* (Townsend) Holland (Burkholder, 1948)) and *Xanthomonas papavericola* (Bryan & McWhorter) Dowson. The pathogen is related to the group of eight Pseudomonads comprising *Pseudomonas calendulae*, *P. cichorii*, *P. nectarophila*, *P. viburni*, *P. mori*, *P. stizolobii* and *P. viciae* which are described as not liquefying gelatin and which are not known to produce acid from sucrose. Within this group it most resembles the descriptions of *P. calendulae*, *P. viburni* and *P. cichorii*, but differs from the first in its lower maximum growth temperature, ability to reduce litmus and inability to produce indole; from the second in its higher salt tolerance and ability to reduce litmus; and from the third in its lower salt tolerance, slower growth on nutrient broth, ability to reduce litmus and non-pathogenicity to lettuce. Our species appears to be sufficiently distinct from other species of *Pseudomonas* to warrant specific rank and we propose that it be named *P. papaveris* sp.nov.



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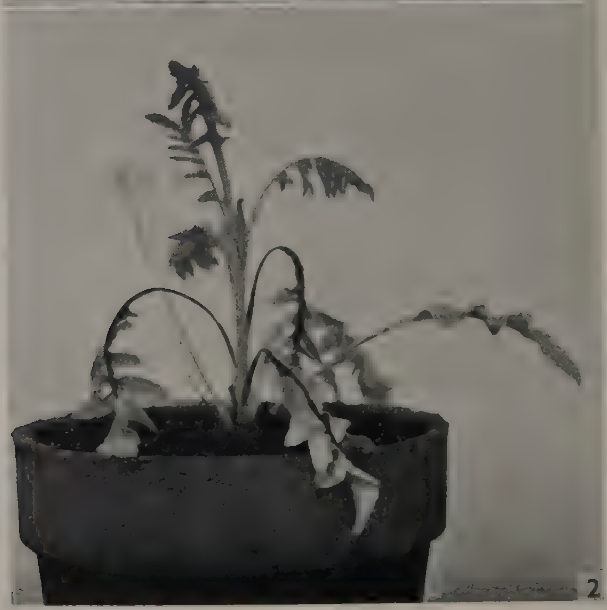

2

(*Facing p.* 91)

Pseudomonas papaveris sp.nov.

Gram-negative, rod-shaped aerobe, 2·0 by 0·4μ (mean), motile with one to several flagella. Eight-day colonies on potato-dextrose agar are whitish, translucent, circular, smooth, glistening, undulate, umbonate and slightly viscid; colonies on beef extract-peptone agar are olive-buff, less undulate and umbonate and produce a yellowish green, fluorescent, water-soluble pigment. Broth turbid with a very light pellicle and a viscid precipitate. Gelatin not liquefied. Litmus milk becomes slowly alkaline with slight reduction at bottom of tube. Maximum salt tolerance 5 %, optimum growth temperature 25–30° C., maximum growth temperature 35° C., thermal death-point 49–50° C. (10 min. exposure). Nitrite not produced from nitrate; indole or hydrogen sulphide not produced; starch partially hydrolysed; not lipolytic. Acid produced from xylose, dextrose, mannose, glycerol, maltose and lactose but not from rhamnose, sucrose, raffinose and salicin; growth but no acid with sorbitol. Causes a disease of *Papaver rhoeas* L. in Tanganyika. It is also pathogenic to *P. orientale* L.

Cultures of the organism have been deposited in the National Type Culture Collection of Bacterial Plant Pathogens, Botany School, Cambridge, and the American Type Culture Collection, Washington.

We wish to thank Dr W. J. Dowson for his helpful advice.

REFERENCES

AYRES, S. H., RUPP, P. & JOHNSON, W. T. (1919). A study of the alkali-forming bacteria in milk. *Bull. U.S. Dep. Agric.* no. 782, 39 pp.

BURKHOLDER, W. H. (1948). In Bergey's *Manual of determinative bacteriology*, 6th ed. Baltimore.

DOWSON, W. J. (1949). *Manual of bacterial plant diseases*, pp. 48–49. London.

MAGOON, G. A. (1926). Studies upon bacterial spores. *J. Bact.* **11**, 253–283.

S.A.B. (1953). *Manual of methods for pure culture study of bacteria.* Edited by Society of American Bacteriologists. Geneva, N.Y.

WALLACE, M. M. (1944). *Mycol. Circ. Tanganyika Territory Dep. Agric.* no. 13, p. 2 (mimeographed).

EXPLANATION OF PLATE 3

Fig. 1. Naturally infected Shirley poppy plant showing stem infection by *Pseudomonas papaveris.*

Fig. 2. Young Shirley poppy plant 6 days after needle inoculation of midribs of three leaves.

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BRITISH RECORDS

Members of the Society frequently record fungi well known, or at least adequately described, elsewhere but hitherto unknown in Britain and British fungi on new hosts or in new localities, and they refind fungi which have not been noticed in Britain during modern times. Such records often do not justify a paper or separate note but they are of interest and deserve being placed on public record to await the next British compilation or revision of the group to which they belong. In order to encourage and give precision to such record-keeping, it is intended, as often as necessary, to include an entry for 'British Records' in these *Transactions*.

Each record submitted for publication under this heading should include the current name of the fungus (with any synonyms), reference to the original description or to the description in the standard work or monograph used when making the identification, the scientific name of the host (which for a British plant should be that in the *Flora of the British Isles*, 1952) or the name of the substrate, the locality (with the National Grid Reference when desirable), the date, the name of the collector and the person making or confirming the identification when not the author of the record, the whereabouts of any specimen or culture, and finally any annotation and line drawings that may be necessary. Each record will appear above the name of the person making the record. No reprints of individual records will be available but each contributor will receive six free reprints of the whole entry of which additional copies may be purchased.

Records for publication may be sent to one of the editors.

(* New British host record. ** New British fungus record.)

1. ***Melanotaenium endogenum** (Unger) de Bary (Ainsworth & Sampson, *Ustilaginales*, 1950, p. 101). On *Galium mollugo* L. [type host for *M. endogenum*], Boxwell, nr. Leighterton, Glos., July 1953. Herb. I.M.I. no. 56758.

LILIAN E. HAWKER, The University, Bristol

2. **Gyrocratera ploettneriana** P. Henn. (Hawker, *Trans. roy. Soc.* B, **237**, 454, 1954). Lanthwaite Woods, nr. Cockermouth, Cumberland, under Douglas Fir (*Pseudotsuga taxifolia*) in needle layer, April 1954. Herb. Bristol University (Collection of hypogeous fungi no. H. 1012) and Herb. Kew.

There are only four previous British records (Beds (2), Surrey, Devon), but a search of suitable habitats may reveal that this fungus is actually not uncommon.

LILIAN E. HAWKER, The University, Bristol

3. ****Polyporus albellus** Peck (*Leptoporus albellus* (Peck) Bourdot & Galzin, *Hyménomycètes de France*, p. 543, 1928).

This interesting species has apparently not been recorded for this country. It is rare, but seven collections have been made during recent years: leg. E. J. H. Corner, Callander, Perthshire, 2 September 1933, on dead *Betula* branches (Herb. Corner no. 695); Brandon, Suffolk, 4 October 1946, on *Betula* (Herb. Corner no. 706): leg. P. D. Orton, Netley Heath, Surrey, 4 November 1950, on *Pinus* stump (Herb. Austwick no. 423); Oxshott Heath, Surrey, 21 October 1951, on birch (?) stump (Herb. Austwick no. 592): Banstead Wood, Surrey, 11 October 1952, on *Quercus* (?) stump (Herb. Austwick no. 742); Deerleap Wood, Wotton Hatch, Surrey, 28 September 1953, on *Corylus* branch (Herb. Austwick no. 817): leg. L. E. Hawker, Denny Park, New Forest, Hants. (B.M.S. Foray), 12 September 1954, on *Betula* twig (Herb. Austwick no. 945).

P. albellus is readily distinguished in the field from *P. lacteus*, and other white polypores, by its white or grey, suede-like surface, and soft, water-soaked flesh. On drying a distinct pellicle appears and the specimen becomes hard and yellow. It is dimitic with binding hyphae giving a complex system of branching hyphae, $3–5\mu$ wide. The basidiospores are allantoid, hyaline, $3{\cdot}5–5{\cdot}0 \times 1{\cdot}5–2{\cdot}5\mu$.

(Herb. Austwick no. 945, has been deposited in the Herb. Kew. and Herb. Austwick no. 817 in the herbarium of the Forest Products Research Laboratory, Princes Risborough, where cultures from Herb. Austwick nos. 592 and 742 are also maintained.)

P. K. C. AUSTWICK, Veterinary Laboratory, Weybridge, Surrey

4. **Psalliota litoralis** Wakefield & Pearson (Pearson, *Trans. Brit. mycol. Soc.* **29**, 205, 1946). In semi-fixed dune, Dawlish Warren, S. Devon, 10 October 1953, leg. T. J. Wallace, det. Miss E. M. Wakefield. Herb. Kew.

First described from collections made by Dr F. R. Elliston Wright in 1944 and 1945 on Saunton Down, N. Devon. This may be the second known locality for this species.

T. J. WALLACE, University College, Exeter

5. ****Inocybe decipiens** Bres. (Heim, *Le Genre Inocybe*, p. 324, 1931). Associated with *Salix repens* in slacks on sand dunes, Braunton Burrows, N. Devon, 10 September 1951; Freshfield and High Town Dunes, Lancs., 5 September 1953, 17 July 1954; Bettyhill dunes, Sutherland, 28 July 1954, det. T. J. Wallace (confirmed by Dr R. W. G. Dennis). Herb. Myc. Exon.

Not recorded in *Revised List of British Agarics and Boleti*, 1948. A rather large *Inocybe* with broadly umbonate brown cap and stout whitish stem. Cap smooth and usually silky at centre and radiately fibrillose at margins. Spores subangular, the nodules indistinct, $9–12\ (–15) \times 6–8\ (–10)\,\mu$. Cystidia marginal and facial, flask-shaped, swollen and with short neck, muricate.

T. J. WALLACE, University College, Exeter

6. **Laccaria trullisata** (Ellis) Peck ssp. **maritima** (Teod.) Andersson (Andersson, *Botaniska Notiser, Suppl.* **2**, 23–27, 1950). In loose sand among scanty *Ammophila* and *Carex arenaria* behind foredunes, Culbin Sands, Morayshire, 17 September 1953, det. T. J. Wallace (confirmed by the late Mr A. A. Pearson and by Dr R. W. G. Dennis). Herb. Myc. Exon.

Mention of this agaric at Culbin was made by Pearson (*Trans. Brit. mycol. Soc.* **26**, 41, 1943) under *Omphalia rosella* J. E. Lange. Superficially very similar to *Laccaria laccata*, but microscopically easily recognized by its large smooth ellipsoid spores 12–17 × 7–8 μ. The only British locality seems to be the acid dunes of Culbin where in spite of afforestation it persists.

T. J. WALLACE, University College, Exeter

REVIEWS

Insect, Fungus and Weed Control. By E. R. de Ong. (London: Thames and Hudson; New York: Chemical Publishing Co. Inc. 1953.) 400 pp., 37 text-figures. Price 52*s*. 6*d*.

This book, which in point of typography and style of presentation might have come from one of our University Presses, provides a compact practical account of the principal fungicides, insecticides and weed-killers now in commercial use in the United States. It deals not only with plant-protection materials, but also with materials used for the control of pests on livestock, household pests, and rodents, and with industrial insecticides and fungicides. There are interesting chapters on modern methods of application, and on the labelling of products under the Federal Insecticide, Fungicide and Rodenticide Acts.

Mycologists and plant pathologists working in this country will perhaps find the book most useful for obtaining a general view of the range of new materials, from aldrin to ziram, that have come into use during the past decade, and for 'refreshing the memory' about their names, nature and uses. Many details of application, and full references, are given, but these almost all concern American practice. More information will often be needed on the *chemistry* of the newer products, but this is already available in Dr Hubert Martin's *Guide to the Chemicals used in Plant Protection*, Ontario, 1953, 295 pp., which is obtainable from the Chief Editor, Information Service, Confederation Building, Ottawa, Ontario (price one dollar).

E. C. LARGE

Grasses: a Guide to their Structure, Identification, Uses and Distribution in the British Isles. By C. E. Hubbard. (A Pelican Book. Harmondsworth, Middlesex: Penguin Books Ltd. 1954.) xii + 428 pp., 158 pages of text-figures by Joan Sampson. Price 3*s*. 6*d*.

This invaluable work includes lists of species according to habitat; a key for naming wild and agricultural grasses; descriptions of British grasses; keys for naming common grasses by vegetative characters and 'seeds'; sections on useful and ornamental grasses, on the structure of grasses, on classification, together with a bibliography and glossary.

Mushroom Science: II: the Proceedings of the Second International Conference on Scientific Aspects of Mushroom Growing, Gembloux, 1953. (Gembloux: Centre de Recherches sur les Champignons, Institut Agronomique de Gembloux, 1954.) 183 pp.

Papers in English and French on many aspects of mushrooms and mushroom growing, including: systematics of *Agaricus*, biochemistry, variation, cultural practices, market problems, composts, spawn and diseases.

Mushroom Research Association Limited: Report for the year 1953. (Yaxley, Peterborough: Mushroom Research Association Ltd., 1954.) 46 pp. Price 5*s*.

This report, like those of previous years, describes progress in research on casing soil; cropping under various treatments; and on parasites and diseases.

The Genetics of Aspergillus nidulans. By G. Pontecorvo. (With sections by J. A. Roper, L. M. Hemmons, K. D. MacDonald, and A. W. J. Bufton.) In *Advances in Genetics*, vol. 5, pp. 141–238. New York, N.Y.: Academic Press Inc. 1953.

In this monograph *Aspergillus nidulans* gains the distinction of being the first homothallic fungus to be analysed genetically. It is discovered that a self-fertile strain may take part in preferential outcrossing, if given the opportunity. Genetic recombination can now be obtained outside the sexual cycle. The monograph provides the background to subsequent work of this school which shows that nuclear fusion, mitotic crossing over and haploidization (the 'parasexual' cycle) occur with frequencies which make this sequence an efficient substitute for sexual reproduction, in a manner which opens up new avenues of thought in academic mycology, in industrial mycology and in plant pathology.

The Rust Fungi (Uredinales) of Gloucestershire. By T. A. Sprague, *Proc. Cotteswold Naturalists' Field Club*, *1952*, **31**, 86–100, 1954.

The records of the 143 species of rusts observed in Gloucestershire, and arranged according to Wilson & Bisby's 1954 check-list, are based on collections made by the author and H. K. Airy-Shaw, a MS. list of Gloucestershire rust fungi compiled by the late H. H. Knight, specimens in the Kew Herbarium, and foray reports in these *Transactions*.

CHEESMAN AND PETHYBRIDGE BEQUESTS

The terms approved by the Council for the administration of these Bequests provide for financial help by the allocation annually of grants to enable one or more students of mycology to attend the Autumn Foray or Plant Pathology Field Meeting, who would otherwise be unable to do so. Applications from students or recommendations from Heads of Departments or members of the Society should reach the Secretary, or the Secretary of the Plant Pathology Committee, not later than 1 May each year.

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13	*m*	*m*	*m*	*m*
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PLANT PATHOLOGY COMMITTEE: *List of Common British Plant Diseases*, 3rd Ed. 1945. Price net, 5*s.* Postage, 4*d.*

A. A. PEARSON: *Cooke's illustrations of British Fungi* (*Annotations by L. Quélet, R. Maire & C. Rea*). *Ex* Vol. 20, Pt. 1. 1935. Price net, 2*s.* 6*d.* Postage, 3*d.*

T. PETCH: *British Hypocreales. Ex* Vol. 21, Pts. 3 & 4. 1938. Price net, 2*s.* Postage, 3*d.*

A. A. PEARSON & R. W. G. DENNIS: *Revised list of British Agarics and Boleti. Ex* Vol. 31, Pts. 3 & 4. 1948. Price net, 5*s.* Postage, 3*d.*

J. RAMSBOTTOM & F. L. BALFOUR-BROWNE: *List of Discomycetes recorded from the British Isles. Ex* Vol. 34, Pt. 1. 1951. Price net, 5*s.* Postage, 3*d.*

FIFTY YEAR INDEX TO THE TRANSACTIONS, Vols. 1–30; 1896–1946. Compiled by G. R. BISBY. Price net, 21*s.* Postage, 6*d.*

E. M. BLACKWELL: Haustoria of *Phytophthora infestans* and some other species. *Ex* Vol. 36, Pt. 2. 1953. Price 4*s.* Postage, 3*d.*

K. SAMPSON & J. H. WESTERN: *Diseases of British Grasses and Herbage Legumes.* 2nd Edition. 1954. Price, 15*s.* Postage, 6*d.*

HILDA M. CANTER: *Annotated List of British Aquatic Chytrids. Ex* Vol. 36, No. 4. 1953. Price 3*s.* Postage, 2*d.*

MALCOLM WILSON & G. R. BISBY: *List of British Uredinales. Ex* Vol. 37, No. 1. 1954. Price 3*s.* Postage, 2*d.*

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FORAY COMMITTEE PUBLICATIONS

(*To be obtained from the Treasurer and on sale at meetings and forays*)

A key to the genera of the agarics and Boleti. By A. A. PEARSON. 32 pp. 1950. Price net, 2*s.* 6*d.* Postage, 2*d.*

Guide to the literature for the identification of British fungi. 1952. Price net, 2*s.* 6*d.* Postage, 2*d.*

Census Catalogue of British Lichens. By W. WATSON. 1953. Price 7*s.* 6*d.*

CONTENTS

PAGE

Presidential Address: Microbial ecology of the soil. By S. D. Garrett. (With portrait) 1

Mycoses of the Belgian Congo. By R. Vanbreuseghem 10

On *Helicodendron tubulosum* and some similar species. By Janet I. Glen-Bott. (With 10 Text-figures) 17

Pythium thalassium sp.nov. infecting the egg-mass of the pea-crab, *Pinnotheres pisum*. By D. Atkins. (With 9 Text-figures) 31

Harposporium crassum sp.nov. By Audrey M. Shepherd. (With 1 Text-figure) . . 47

The cytology and relationships of *Corynebacterium fascians*. By Margaret S. Lacey. (With Plate 1 and 4 Text-figures) 49

Fairy ring kinetics. By A. F. Parker-Rhodes. (With 2 Text-figures) . . . 59

Hypogeous fungi. IV and V. By Lilian E. Hawker. (With 2 Text-figures) . . 73

The effect of trace metal deficiency on some *Trichophyton* strains. By Mary P. English and N. H. Barnard. (With Plate 2) 78

A selective medium for the enrichment culture of *Pseudomonas mors-prunorum* Wormald. By J. E. Crosse and Margery Bennett 83

A bacterial disease of Shirley poppies in Tanganyika. By R. A. Lelliott and Maud M. Wallace. (With Plate 3) 88

British Records 92

Reviews 95

Cheesman and Pethybridge Bequests 96

INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and subheadings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, Cambridge University Press, 200 Euston Road, London, N.W. 1, price 2*s*. 6*d*.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

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